EIMERIA TENELLA: COMPARATIVE PATHOLOGY AND LESIONS OF EXPERIMENTAL INFECTIONS IN BACTERIA-FREE, SPECIFIC PATHOGEN-FREE AND CONVENTIONAL CHICKENS

By

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To my parents Subhalakshmy and Venkitasubban who inspired me to pursue a scientific career.

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In order to determine the role, if any, of the indigenous cecal microflora of chickens in influencing the development of and disease due to Eimeria tenella, the pathology and lesions following experimental inoculation with a standard dose of E. tenella infective oocysts in bacteria-, fungi-, pleuropneumonia-like organisms-free (PPLO-free), specific pathogen-free (SPF) and conventional chickens were studied.

The dominant microflora of apparently healthy conventional chickens and changes in the indigenous flora following infection with E. tenella were studied in chickens aged 1, 7, 14, 21, 28, and 35 days, using standard microbiological procedures. Experimental exposure was carried out by oral inoculation with 100,000 surface-sterilized E. tenella oocysts alone or combined with single or multiple species of bacteria and/or fungi.

No clinical symptoms, mortality or gross lesions

were observed in a total of 32 bacteria-, fungi- and PPLO-free chickens inoculated with E. tenella alone. In these hosts a retardation of the development of the endogenous stages of E. tenella was evident. The presence of a pure strain of Bacteroides sp., Clostridium perfringens, Escherichia coli, Lactobacillus sp. or Streptococcus fecalis or single species of the fungi Candida albicans or Mucor sp. resulted in mild cecal coccidiosis following inoculation with E. tenella. The typical cecal coccidiosis syndrome developed in chickens harboring 2 or more species of microflora, viz., C. perfringens and S. fecalis; E. coli and S. fecalis; Bacteroides sp., C. perfringens, E. coli and S. fecalis. In SPF chickens, typical cecal coccidiosis developed following experimental infection, with a mortality rate of 38% and a mean gross lesion score of 2.6. In conventional chickens, the mortality rate was 22.1% and the mean gross lesion score was 3.1. C. perfringens was isolated more frequently from noninfected SPF chickens than from noninfected conventional chickens. A stimulation of growth of C. perfringens and coliforms occurred with a concomitant reduction in the growth of Lactobacillus sp. in SPF and conventional chickens suffering from typical cecal coccidiosis.

The results indicate that certain species of the indigenous microflora of the ceca are essential to produce the typical cecal coccidiosis syndrome, following ingestion of E. tenella infective oocysts.

INTRODUCTION

Eimeria tenella [Railliet and Lucet, 1891; Protozoa: Eimeriidae] is the most common and pathogenic of the 9 species of Eimeria described from the chicken (Gallus domesticus). All the 9 species of Eimeria occurring in the chicken are intracellular parasites of the epithelial cells of the intestinal tract producing the disease known as coccidiosis.

Coccidiosis is a disease of great economic importance to the poultry industry throughout the world. In the United States alone, a total loss to the poultry industry of $34,854,000 was estimated during the period 1951 to 1960 by the United States Department of Agriculture (1965). Of this sum, $15,123,000 were attributed to mortality and $19,731,000 to morbidity. In 1966, the expenditure for coccidiostatic drugs in the United States was estimated to be between $40,000,000-$50,000,000 for broiler and laying flock replacement chickens. Acute coccidiosis with a high rate of mortality is regularly associated with infection due to E. tenella and since the lesions are confined to the ceca, the disease is often referred to as acute cecal coccidiosis. Young chickens 3-8 weeks of age are highly susceptible to this disease with a peak susceptibility at

about 4 weeks of age (Gardiner, 1955). Several other authors also suggest that young chickens are more susceptible than older ones (Tyzzer, 1929; Karmann and Presch, 1933). Conversely, many others suggest that the reverse is true (Tyzzer et al., 1932; Mayhew, 1934; Jones, 1932; Horton-Smith, 1947). It is important to distinguish between susceptibility of chickens to clinical disease and susceptibility to coccidial infection as measured by oocyst production. Based on oocyst production, older birds are more susceptible to the parasite than the younger ones (Rose, 1967b). This is due to the higher rate of excystation of the oocysts (Doran and Farr, 1965; Rose, 1967b). Older birds are also susceptible to clinical infection but the rate of mortality is usually low due to an apparent acquired immunity after previous nonfatal exposures. Levine (1963) suggested that the acquired immunity is often not absolute, but generally only a condition of relative immunity. One-day-old or 1-week-old chickens are less susceptible to the infection when compared to chickens 3-5 weeks of age (Rose, 1967b). Thus the severity of the infection and disease in chickens under field conditions is related to age and breed of the chickens, previous exposure, and the degree of exposure to the infective stage of the parasite. At the present time, coccidiosis is controlled by the routine use of various coccidiostatic drugs. For practical and economic reasons these drugs have

to be incorporated in the feed or drinking water of the chicken from the day of hatching and continued throughout the life of the bird. This continued use of drugs has resulted in interference with immunity (Davies and Kendall, 1955; Reid, 1960), side effects such as reduced fertility (Joyner, 1964), and development of drug-resistant strains (McLoughlin and Gardiner, 1961a, 1961b, 1962; Pellérdy, 1961, 1962a, 1962b; Gardiner and McLoughlin, 1963; Vegh, 1963; Joyner, 1970; McLoughlin, 1970). Moreover, drugs presently available do not offer effective protection against all the species of Eimeria parasitic in chickens and most of the current broad spectrum coccidiostats are not suitable for prolonged periods of use in chickens intended for human consumption. In spite of the high efficacy of modern coccidiostats, outbreaks of the disease may occur (Joyner, 1970) due to high levels of contamination in the environment, reduced uptake of the drug or development of drug-resistance, and a high degree of susceptibility (Joyner, 1964, 1970).

Under natural conditions, cecal coccidiosis occurs through ingestion of large numbers of the infective stage of E. tenella called sporulated oocyst. The unsporulated oocysts which are formed in the epithelial cells of the ceca are expelled with the feces. They develop and sporulate on the ground if conditions of oxygen tension, moisture, temperature, and other environmental factors are suitable.

The sporulated oocyst, containing 8 sporozoites, is the infective stage. The first step in the pathogenesis of the disease is excystation of the ingested oocysts. Rose (1967b) found rapid excystation of the majority of the oocysts in highly susceptible chickens 4, 5, or 6 weeks old, while less successful excystation and low oocyst production occurred in 0-3-week-old chickens which are also less susceptible to cecal coccidiosis. She ascribed the reasons for very low percentage of excystation to immaturity of the hosts (weak action of the gizzard wall and sub-optimal concentration of tryptic juices). There are a combination of factors necessary for excystation such as bile and pancreatic juice (Levine, 1942; Ikeda, 1956; Hibbert et al., 1969).

Our knowledge regarding the role of intestinal flora in the initiation, development, or severity of cecal coccidiosis is limited. However, it has been reported by Johansson and Sarles (1948) that during E. tenella infection the growth of Clostridium perfringens is stimulated while growth of Lactobacillus sp. is suppressed. The cecal bacterial flora constitutes 90% of the total gastrointestinal flora and is of great biological importance to the health of chickens for synthesis of certain vitamins (Coates et al., 1968; Timms, 1968).

The effect of normal bacterial flora on the biology and immunology of the host and possible relationship between

this flora and certain diseases have been investigated by many workers. Phillips et al. (1955), using germ-free guinea pigs, proved that presence of bacterial flora is essential for survival of Entamoeba histolytica and pathogenesis of amoebiasis. Based on these findings, Wittner and Rosenbaum (1970) studied the role of bacteria in modifying the virulence of E. histolytica. Bradley and Reid (1966) demonstrated a dual etiology involving a protozoan (Histomonas meleagridis) and a single species of bacteria (Escherichia coli, C. perfringens, or Bacillus subtlis) for infectious enterohepatitis in turkeys. Several other studies have been made on the role of bacterial flora affecting the course of infection in infectious enterohepatitis (Doll and Franker, 1963; Franker and Doll, 196-, Reid et al., 1969; Springer et al., 1970). Hegde et al. (1969) studied the pathogenicity of E. brunetti in bacteria-free chickens and showed that the parasite can develop and produce disease in bacteria-free chickens. No substantial studies, however, have been made as to the possible role of bacteria or other microflora in relation to pathology due to E. tenella using gnotobiotic (bacteria-, fungi- and pleuropneumonia-like organisms (PPLO)-free) chickens. In preliminary studies, Clark et al. (1962) found no difference in the course of E. tenella infection in bacteria-free and conventional chickens. There was, however, a delay of 12 to 15 hours in the appearance of the 2nd generation

merozoites in the feces of gnotobiotic chicks. Visco and Burns (1966, quoted by Hegde et al., 1969) reported a close relationship between the host microflora and E. tenella in the production of the cecal coccidiosis syndrome. As Hegde et al. (1969) reported, "the effects of the bacterial flora on the pathogenicity of this species remains unsolved." It is not clear whether the bacterial flora hinders or aids E. tenella to initiate and develop the disease entity. The present study was undertaken to find out the influence, if any, of the microbes normally present in the ceca of chickens in the development of cecal coccidiosis, by determining the ability of E. tenella to produce the specific pathology and lesions of cecal coccidiosis in chickens harboring no detectable microorganisms and by comparing the pathology of experimental infection with E. tenella in bacteria-, fungi-, and PPLO-free chickens, specific pathogen-free chickens and conventional chickens. To prove Koch's postulates with regard to cecal coccidiosis bacterial isolates from conventional disease-free chickens and chickens showing typical lesions of cecal coccidiosis were also compared. Bacteria-, fungi-, and PPLO-free and SPF chickens were inoculated with standard doses of E. tenella sporulated oocysts isolated from naturally-infected cases, either alone or combined with bacterial species. Any organism or combination of organisms capable of producing cecal coccidiosis in gnotobiotic chickens were then

isolated and the isolates used for further inoculation of conventional, SPF and bacteria-free chickens. The knowledge of the interrelationship between the normal microbial flora of the ceca and E. tenella gained by the present and subsequent studies may ultimately lead to better means of control of cecal coccidiosis.

LITERATURE REVIEW

Eimeria tenella [Railliet and Lucet, 1891] is a protozoan belonging to the Family Eimeriidae, Class Sporozoa. The term "coccidia" is generally used to describe species belonging to the Family Eimeriidae (Becker, 1934). During the last 10 years, studies on the fine structure of coccidia and related groups have revealed a great number of new similar structures namely the pellicle, the polar rings, the subpellicular microtubules, the rhopteries, the micronemes, the micropore and the cono... These fine structural similarities are considered as indication of a close relationship and Levine (1969) proposed a slight modification of this classification and Scholtyseck and Mehlhorn (1970) have discussed the problems of taxonomy of Sporozoa. In the domestic chicken (Gallus domesticus), 9 species of the genus Eimeria, E. acervulina, E. brunetti, E. hagani, E. maxima, E. mitis, E. mivati, E. necatrix, E. praecox, and E. tenella, have been described as parasites of the epithelial cells of the various regions of the intestinal tract (Biester and Schwarte, 1965). E. mivati is the only species which may be found in several regions of the intestinal tract (Edgar and Seibold, 1964). The 9 species can be differentiated by morphological characteristics, sporulation time of the oocysts, developmental

features, localization in the host, and degree of pathogenicity. Of the morphological characters, the structure of the oocyst is usually used to identify the species at least within a given host (Levine, 1961), but oocyst characters alone have only limited value in differentiating species of Eimeria (Horton-Smith and Long, 1963).

Among the many means of biological differentiation for Eimeria species, the location of endogenous stages in the specific region of the intestinal tract of the host and species specific immunity are of major importance. Each species shows a marked regional specificity (Tyzzer, 1929; Tyzzer et al., 1932; Herrick, 1936) for the development of endogenous stages. Also infection with a species results in immunity against that species but not against others even within the same host. Hence cross-immunity tests can be used to differentiate the various species of coccidia (Tyzzer, 1929; Tyzzer et al., 1932) but the specificity of acquired resistance may not be rigid. Rose (1967a) found cross-immunity between E. tenella and E. necatrix when using sporozoites of E. necatrix to induce infection in the cecum. Therefore a combination of factors is always used for species identification.

Eimeria tenella: Life Cycle and Morphology

Acute cecal coccidiosis in young chickens is regularly associated with E. tenella and this species is the most common and most pathogenic of all coccidia found in

chickens (Davies et al., 1963). Tyzzer (1929) published a detailed description of the morphology and life cycle of E. tenella which has been confirmed in all details by subsequent investigation (Edgar, 1941). Like other species of Eimeria, asexual and sexual generation occur in the same host following ingestion of viable sporulated oocysts through food and/or water. Through a process of excystation, sporozoites escape from the sporocysts and oocysts, but the factors contributing to excystation have not been definitely established. Studies by Levine (1942) and by Ikeda (1955a, 1955b, 1956, 1960) revealed that pancreatic juice, in particular trypsin, is one of the factors responsible for excystation. Goodrich (1944) observed the escape of sporozoites through any available fracture in the cyst wall 5-10 minutes after the cyst wall has been placed in a 5% trypsin solution, maintained at 37° C. Hydrogen-ion concentration, bile and buffers were also found to be important factors in excystation of various species coccidia (Smetana, 1933; Lotze and Leek, 1960; Doran and Farr, 1962; Nyberg and Hammond, 1964). Hibbert et al. (1969), studying the effects of pH, buffers, bile and bile acids on the excystation of sporozoites of various Eimeria species including E. tenella, found no excystation when any of the bile acids or bovine or chicken bile was used alone without trypsin. However, they observed excystation of E. bovis and E. ellipsoidalis in bovine bile containing a heavy suspension of bacteria and fungi. When

trypsin alone was used only E. bovis oocysts excysted, the other 9 species of Eimeria including E. tenella did not excyst. The precise way in which bile acts in excystation of oocysts is not known at present. Doran and Farr (1962) suggested that bile acids may alter the protein or lipoprotein surface of the steidae body in such a way that it is then readily acted upon by pancreatic enzymes and/or may facilitate entrance of enzymes into the intact oocysts through the altered micropyle. Lotze and Leek (1969) found that in adult chickens about 40-60 minutes may be required for E. tenella oocysts to be carried from the mouth to the large intestine. A permanent opening or micropyle was not observed in the oocyst wall through which sporozoites might escape. Therefore the release of large numbers of sporozoites into the digestive tract of chickens requires the wall of the oocyst to be broken, weakened or partially dissolved. Lotze and Leek (1969) observed the walls of many sporulated oocysts expelled through feces to be structurally changed. Development of immunity does not hinder excystation and normal excystation will occur in immune and non-immune chickens under optimal conditions (Horton-Smith et al., 1963).

Liberated sporozoites are fusiform, 10 μ x 1.5 μ in diameter, transparent and motile. Each sporozoite has a nucleus, a prominent refractile globule at the rounded end, and exhibits various types of movement. They rapidly invade

the surface epithelial cells of the cecum and then penetrate the basement membrane to enter the tunica propria through which they either pass free or within the macrophages, to finally reach the epithelial cells lining the fundus of the Lieberkühn glands where asexual reproduction by schizogony occurs (Challey and Burns, 1959; Pattillo, 1959). After entry into a glandular epithelial cell, the sporozoite rounds up and becomes a trophozoite which develops into a 1st generation schizont (24 μ in diameter) within 24-48 hours. The nucleus of the invaded cell becomes hypertrophied (Levine, 1963) and the parasitized cell bulges out into the cecal lumen. Each schizont forms about 900 merozoites (Tyzzer, 1929) 2-4 μ in length 1-1 [illegible] in width. Merozoites after release from the mature 1st generation schizonts enter a new host cell by direct penetration and migrate into subepithelial layers of the tissue to develop as 2nd generation schizonts. The ultrastructure of merozoites and the fine structural changes have been described by McLaren and Paget (1968) and McLaren (1969). Growth of the 2nd generation schizonts is rapid and within 24 hours mature schizonts, containing numerous merozoites, can be observed. The large 2nd stage schizonts of E. tenella are found in the epithelial cells which appear to have moved from the epithelial layers into the subepithelial layers, submucosa and even into the muscular layers of the ceca. The maturation and release of large numbers of 2nd

generation merozoites causes extensive destruction of the epithelial cells and severe hemorrhage occurs into the cecal lumen followed by tissue necrosis and thickening of the cecal wall by the 4th and 6th day. The 2nd generation merozoites are considerably larger than the 1st, averaging about 16 μ in length, 2 μ in width and 200 to 350 in number, many of which enter new host cells and begin the sexual phase of life cycle by developing into either macrogametocytes or microgametocytes. Microgametocytes are smaller both in size and numbers than macrogametocytes and the 2 are found in close proximity within columnar epithelial cells of the ceca, below the host cell nuclei. From each microgametocyte, microgametes develop. Each microgamete has 3 flagella and is motile.

Young macrogametocytes are large irregularly shaped cells measuring approximately 5.3 μm x 7.5 μm. Though the young macrogametocyte still retains the shape of the merozoite, it can be distinguished from schizont or microgametocytes by the presence of "wall forming" or membraneous bodies (Scholtyseck, 1962) under the electron microscope. Later "dark bodies" develop which are thought to correspond to the "plastic granules" described from light microscope investigations (Reich, 1913; Doflein and Reichenow, 1953; Cheissin, 1958). After fertilization these bodies migrate to the periphery of zygote. The limiting membranes of the zygote then separate from the cell to become the outermost

membrane of the oocyst wall. The middle layer of the cyst wall is developed from the "dark bodies" and the "wall forming bodies" give rise to the inner layer of the oocyst wall. Thus, in E. tenella the oocyst wall is trilaminate. When the oocyst wall is complete the oocyst is extruded from the host tissues and is passed to the exterior with the feces. The period from the time of infection to the 1st appearance of oocysts is usually 7 days. The oocyst production thus commences on the 7th day following exposure, reaches a peak by the 10th day and rapidly decreases. Oocysts of E. tenella are ovoidal, clear, transparent, with a well defined double outline. The outer layer of the oocyst wall is quinone tanned protein and the inner layer is a lipid coat firmly associated with a protein lamella (Honné and Hönig, 1954). The size of the oocysts range from 14.2 μ to 31.2 μ x 9.5 μ to 24.8 μ, with a mean of 22.96 (± 2.2) x 19.16 (± 1.69) μ (Becker, 1956). The optimum temperature for sporulation of the oocysts is 29 ± 1° C. (Edgar, 1955) and at this temperature sporulation will be completed in 18 hours but at room temperature it takes about 48 hours. The sporulated oocysts contain 4 sporocysts each containing 2 sporozoites. Like in other species of Eimeria meiotic division occurs during sporogony. E. tenella can be cultivated in the developing chick embryo (Long, 1965, 1966, 1971) and in tissue culture cells (Patton, 1965; Bedrnik, 1967, 1969; Strout and Ouellette,

1969; Matsuoka et al., 1969; Doran, 1970), using sporozoites obtained by in vitro excystation.

Pathogenesis of E. tenella Infection

Factors affecting pathogenicity of E. tenella include the number of oocysts ingested, the number of host cells destroyed per ingested oocyst, the degree of re-infection and the state of immunity in the host. The severity of the disease depends upon the interplay of these known and other unknown factors and range from an imperceptible reaction to death (Gardiner, 1955). Cecal coccidiosis under field conditions occurs principally in young chickens but seldom in those less than 10 or 11 days old. The range of age of susceptibility is from 2 weeks to 15 months. Many of the worst outbreaks occur at the age of 6 to 8 weeks (Biester and Schwarte, 1965). Herrick et al. (1936) in a study on experimental infection found that the heaviest mortality and greatest decrease in erythrocytes occurred in chicks 1 month old; heavy mortality also occurred in chicks aged 2 weeks and 2 months while in older birds (3, 4, 7, 10, and 15 months of age) mortality was low or lacking though drop in red cell count ranged from 29% to 46.8%. Gardiner (1955) employing a dosage of 50,000, 100,000, and 200,000 sporulated oocysts infected young chickens in age groups of 1, 2, 3, 4, 5, and 6 weeks. Those in the 4 week group were severly affected and those in the 2 week group were least affected. Those birds which

recover from infection become immune to reinfection with E. tenella. However, this is not an absolute immunity. Under conditions of stress, the acquired immunity of older birds may break down causing symptoms of the disease to reappear (Levine, 1963). Levine (1940) in a study of subclinical coccidial infection in pullets at least 8 months old reported the presence of E. tenella in 23%. In general, however, it can be shown that chicks rigidly isolated from infection remain fully and uniformly susceptible throughout their lives and that age per se has no influence on resistance. Under field conditions almost all chickens get early exposure to at least light infection and so nearly all chicks more than a few days old have some degree of resistance.

Inherited resistance in some strains of chickens to infection with E. tenella has been reported by Rosenberg (1941) and Rosenberg and McGibbon (1948) but in general there is little evidence of any significant variation in susceptibility between different breeds or strains of chickens (Horton-Smith and Long, 1963). Jeffers and Wagenbach (1969) reported higher susceptibility and mortality of female chick embryos from widely different genetic sources to E. tenella infection. Edgar and Herrick (1944) produced evidence to show that the presence of food in the digestive tract of birds at the time of infection reduced the severity of the disease. Holmes et al. (1937) suggested

that increased death rate may result in chickens having higher amount of oyster shells in the ration. The number of oocysts resulting from an infection is not a true indication of the degree of infection. Tyzzer (1929) postulated that, theoretically, infection with a single oocyst of E. tenella could give rise to approximately 1,800,000 oocysts in a period of 4 to 5 days. During the course of an infection, however, there are several factors which may cause a reduction in this potential including loss of merozoites, over crowding, and tissue damage which results in a loss of suitable cells. Brackett and Bliznick (1949, 1952) reported that for each oocyst of E. tenella inoculated in light infection approximately 10,000 oocysts are produced, and there is no direct correlation between the size of infective dose and the final degree of infection. However, Johnson (1927) reported that the severity of cecal coccidiosis depends on the number of sporulated oocysts that the bird receives. Jankiewicz and Scofield (1934) reported that a dosage of up to 150 sporulated oocysts produced neither symptoms nor mortality; 150 to 500 oocysts produced slight hemorrhage and no mortality; 1,000 to 3,000 oocysts a fairly heavy degree of hemorrhage and moderate mortality and over 5,000 oocysts produced severe hemorrhage and high mortality. The prepatent period in E. tenella infection is 7 days but the patent period varies with individual infections. Fish (1931) reported

that oocysts were not present in the droppings of the infected birds after 17 days although Tyzzer et al. (1932) recorded oocyst passage for as long as 19 days post-infection. The greatest numbers of oocysts are discharged in a very short time (Tyzzer et al., 1932), the few remaining being trapped either in the tissues, or in the cecal contents, and irregularly released. Under natural conditions, birds are usually infected repeatedly and thus may pass oocysts for long periods of time. For example, Levine (1940) observed oocysts of E. tenella in the droppings of 9 out of 30 birds which did not show any symptoms of infection.

The disease symptoms in cecal coccidiosis are closely related to the course of infection and, in general, the degree of pathogenicity is related to the depth to which the cecal wall is parasitized. E. tenella penetrates deeply and is very destructive. During the development of the parasites there is a migration of parasitized cells into the subepithelial region where they increase enormously in size. Much tissue is destroyed and sloughing of mucosa occurs at the time of maturation of 2nd generation schizonts as early as the 96th hour after infection and profuse hemorrhage occurs due to mechanical damage to the blood vessels. This bleeding is the most important effect of the parasitism. Mortality is likely to be great when profuse and continuous bleeding occurs from the 4th to 7th days post-exposure. Much of the damage may also come from

secondary bacterial infection of the area in which the epithelium is completely destroyed (Briggs, 1968). Hemorrhage is a great stress on the infected chicken and feeding and movement are at a minimum during this period, but consumption of water is increased 2 to 3 times that seen in uninfected birds. In a typical severe infection, bloody droppings will occur 96 hours post-exposure and passage of large quantities of blood in the droppings on the 5th and 6th day post-exposure occur. The disease is at its peak on the 7th day post-exposure and 90% of the mortality occurs within 9 days following initial exposure to oocysts. Chickens surviving 9 days following exposure will usually recover. A chronic condition, however, may occur as the result of retention of a core of necrotic tissue in the ceca with consequent cecal dysfunction. In the flock as a whole, the disease is nearly always of short duration. It is often found that a condition of disease arises only when infection, heavy in relation to the previous experience of the birds, is acquired during a period of 72 hours or less. If infection is picked up more slowly, then the birds become resistant before clinical effects appear. When chickens are raised on deep litter as in most parts of the world, the oocysts are not necessarily destroyed by the heat of fermentation, but due to the unfavorable environment are predominantly unsporulated. Changes in the environment, notably an increase in moisture and/or temperature,

favor a high and rapid rate of sporulation leading to a clinical disease in the flock.

Appearance of fresh blood in the droppings and sudden death are of diagnostic value in cecal coccidiosis. Clotting of blood is prevented during the acute stages by some unknown factor(s) (Davies et al., 1963) and deficiency of vitamin K increases pathogenicity to E. tenella and E. necatrix (Davies et al., 1963). Blood drops can be expressed from the vent of dead birds picked up within a few hours. At necropsy, blood-filled ceca and presence of developmental stages of E. tenella can confirm the diagnosis of cecal coccidiosis. The mere presence of oocysts is not indicative of disease since in E. tenella infections, oocysts are not ordinarily seen in an infection sufficiently acute to cause disease and death (Davies et al., 1963).

For experimental infections of coccidiosis, known numbers of sporulated oocysts are administered orally. However, Davies and Joyner (1962) and Sharma and Reid (1964) succeeded in producing cecal infections by introducing E. tenella oocysts subcutaneously, intravenously, intraperitoneally or intramuscularly in chickens. When viable sporozoites were introduced by the same routes, light infections were produced. The method of excystation and transfer to the site of infection for chicken coccidia inoculated parenterally is not yet understood. Horton-Smith and Long (1963) confirmed these findings partially in that

although they could obtain oocysts in the ceca after intravenous and intramuscular administration of oocysts, they failed to recover oocysts from ceca when chickens were inoculated intraperitoneally or subcutaneously. They assumed that oocysts inoculated into the blood stream would be removed from the circulation by the liver along with other foreign bodies. They reported the presence of disintegrating oocysts in the liver of chickens inoculated intramuscularly and suggested that the sporocysts and sporozoites might reach the intestine and ceca via the bile duct. Patnaik (1966) reported that when oocysts were placed in Millipore chambers grafted within muscles, excystation took place inside the chamber with the help of enzymes produced by the infiltrating leukocytes. He also found engulfment of sporozoites by macrophages inside the chamber and postulated that they might carry the spor to various parts of the body. In all these cases much lighter infections occurred after parenteral inoculation compared with the infections occurring after oral inoculation (Horton-Smith and Long, 1963).

Pathology of Cecal Coccidiosis

Involvement of the ceca rather than of the small intestine is one characteristic of E. tenella infection. However, if the ceca are surgically removed or, in very heavy infections, the terminal portion of the large intestine will be parasitized. The lesions associated with

E. tenella infection in the ceca have been described by Tyzzer (1929), Tyzzer et al. (1932) and Mayhew (1937). The dilated part of the cecum is primarily involved and substantial damage is due to the large numbers of relatively large 2nd generation schizonts present in the deeper lamina propria of the mucosa. Production of specific toxin has not yet been demonstrated although parenteral administration of extracts of oocysts is lethal to rabbits and not to chickens (Burns, 1959). The pathologic effects of the maturation of schizonts and release of 2nd generation merozoites are hemorrhage and sloughing of the epithelial lining of the cecum, which is sometimes stripped down to the base of the submucosa and its replacement by a core composed of necrotic tissue, coagulated blood, cecal contents, and developmental stages of the parasite, chiefly oocysts. This core is at first adherent to the cecal wall, but later may get detached and lie free within the lumen. An infected bird may pass this core or a blood clot in the droppings. The cecum of recovering birds may regain its gross appearance but remain slightly thickened. In lighter infections, recovery is often complete and rapid, but in heavier infections, recovery is slow and the mucosa often show only incomplete regeneration. The continuous hemorrhage from the 4th to 7th day post-exposure results in profound anemia which is often the cause of death. The exposed skin and mucous membranes

become pallid. Erythrocyte counts and hematocrit decrease to about 50% of the normal on the 5th and 6th day after infection with 50,000 E. tenella oocysts, and the values return to normal in about 8 days (Natt and Herrick, 1955). Natt (1959) observed lymphocytopenia and heterophilia on the 5th day and an eosinophilia on the 10th day following infection with E. tenella. There were no significant changes in the monocyte and basophil numbers during the course of infection. A marked leucocytosis began on the 7th day post-infection and persisted through the recovery phase of the disease. Pratt (1940, 1941) observed an increase in blood sugar during the acute stages of the disease with a decrease in the muscle glycogen. Waxl (1941) also found a rise in blood sugar on the 5th day post-infection and a rise in blood chlorides on the 6th and 7th days post-infection with accompanying reduction in muscle chloride. However, Freeman (1970) found no hyperglycemia or change in hepatic glycogen, but he noted a significant reduction in the plasma lactate concentration on the 1st and 2nd days after exposure and a rise in cardiac glycogen on the 5th day post-infection. According to Daugherty and Herrick (1952), during the acute stages of the infection, a substance produced in the cecum reduced the ability of the brain of the chicken to utilize glucose but not hexose diphosphate. Thus, in cecal coccidiosis a severe interference with normal phosphorylative carbohydrate utilization may occur. Chalfey (1962) noted an

increase in adrenal ascorbic acid and adrenal corticosterone concentrations during the acute hemorrhagic phase of the infection. Bertke (1963) found renal clearance of uric acid in chickens infected with E. tenella greatest at 2 to 4 days after infection. This study suggested that death is not due to cecal tissue destruction per se nor is it entirely due to cecal bleeding, but to the failure to recover from an initial shock resulting from the development of large numbers of endogenous stages. It is also reported by Johnson and Reid (1970) that in some cases the gross lesions in the ceca in live birds will be more severe than those in dead birds. Musajev and Surkova (1970) studied the nitrogen metabolism of chickens infected with E. tenella and noted that the total and protein nitrogen decrease within the categories of all ages, on the 3rd and especially on the 5th day and this coincides with the period of development of endogenous stages of the parasite. They assume that these disorders are associated with many factors, such as disorders of fermentation and suction and influence of metabolic product of the parasite. They concluded that on the basis of protein metabolism in the liver, deeper pathological changes occur in young chickens than in older birds.

Early work by Levine and Herrick (1954, 1957) showed that the voluntary muscles in infected birds are unable to do more than 50% of the work done by muscles of

uninfected birds when stimulated via the nerves. However, Freeman (1970) found that when the muscles are directly stimulated they are able to do work and speculated that an impairment of nervous conduction at the neuro-muscular junction occurred in coccidiosis.

As for cellular responses, Pierce et al. (1962) showed that during the primary infection with E. tenella, heterophilpolymorphonuclear cells infiltrate into the submucosa in increasing numbers especially on the 4th or 5th day post-exposure when the 2nd generation schizonts are developing and maturing. Lesion scoring has been frequently used to compare quantitatively the extent of gross lesions and pathology. Herrick et al. (1942) first described a method of scoring E. tenella lesions using a 0 to 4+ scoring system. This scoring system has been followed by many workers (Ripsom and Herrick, 1945; Gardiner and Farr, 1954; Cuckler, 1957; Bankowski et al., 1959; Horton-Smith et al., 1961; Lynch, 1961; Britton et al., 1964; Turk and Stephens, 1967; Dunkley, 1968). A modification of this was also used by Cuckler et al. (1958), Waletzky et al. (1949-1950), Ball (1959), Boney (1948), Farr and Wehr (1945), Levine and Barber (1947), Waletzky and Hughes (1949-1950). Horton-Smith et al. (1961) used a system for macroscopic grading of lesions and correlated this by microscopic grading of parasitism, depending upon the presence of endogenous stages. Johnson and Reid (1970) used a grading system for gross pathology

supplemented by examination for developmental stages in the cecal contents. Lesion scoring is time consuming. Should other disease conditions such as ulcerative enteritis appear in the pens, more extensive microscopic studies may be required to decide whether lesions are induced by coccidiosis. Norcross and Washko (1970) examined intestinal tissues from 734 cases of clinically diagnosed or suspected cases of coccidiosis histologically and confirmed coccidiosis only in 58.2% of the cases. No specific pathological manifestations could be found in the intestinal tissues of 28.6% of the cases. The remaining cases were diagnosed in descending order as ulcerative enteritis, leukosis or Marek's disease, other enteritides, helminthiasis and histomoniasis. These studies stress the necessit of both macroscopic and microscopic examination of th intestinal tissues for confirming pathology due to E. tenella infection.

Microflora and Hosts

In nature, animals live in intimate contact with many microorganisms. The microorganisms are thus found either in the immediate environment, on the superficial tissues, or in the gastrointestinal tract of animals. This close association has led, in many cases, to symbiotic relationships between the host animal and the microorganisms. The apparently healthy laboratory and other animals used to study many biological processes carry

their indigenous microflora and these animals are known as "conventional" animals. In contrast, a "germ-free" animal is one from which it is not possible to recover any viable organism. Many laboratories employ tests to detect bacteria, fungi, helminth parasites, PPLO, and certain viruses to determine the germ-free state of the animals (Newton, 1965). The term "gnotobiote" (Reyniers et al., 1949) is also used in referring to the germ-free animal and also animals carrying known species of organisms. A "specific pathogen-free" (SPF) animal is one free of specified microorganisms and parasites known to cause disease (Sabourdy, 1965). The SPF animals are functionally and structurally identical with their conventional counterparts but their flora and fauna are, to some extent, controlled. The production of germ-free or SPF animals is a problem if the particular animal species have certain congenital infections. Salmonella pullorum in chicks and Toxocara canis in dogs (Reece et al., 1968; Griesemer et al., 1963) are two infections transmissible congenitally. Careful selection and isolation of breeding stock free of these infections and prompt elimination of young animals or chicks showing any congenital infections are necessary to insure absence of such infections in the breeding stock (Reece et al., 1968). These elaborate procedures to establish and maintain germ-free or specific pathogen-free animals for research purposes will eliminate the necessity for using experimental animals of unknown disease exposures,

age, breeding and most important environmental background. The possible influence of the "macro" and "micro" environment in disease processes can be studied when a stock of animals derived from a breeding colony is raised under 3 different environments--conventional, SPF and germ-free.

A number of workers have attempted to raise germ-free animals since Pasteur's speculation in 1885 that the host-microflora relationship is obligate. But the investigations of Schottelius (1899), Cohendy (1912), Cohendy and Wollman (1914), Küster (1912), Glimstedt (1936), Balzam (1937a, 1937b), Reyniers (1946, 1949, 1960), Gustafsson (1948) and Miyakawa (1954) proved Pasteur's original assumption wrong. Now the germ-free animal has become a very useful tool for studying true homeostasis of the gnotobiotic host, the individual activities and interactions of microorganisms and the response of the host to these organisms. These interactions have not yet been well defined although a few significant microflora-host relationships have been established.

The Germ-Free Chick

The embryo of healthy birds is maintained in a germ-free condition within the shell until hatching. This has enabled germ-free chicks, turkeys and other birds to be obtained with relative ease. Germ-free chickens are very popular as experimental animals and have been

successfully used to study such research problems as the origin of blood group B agglutinins (Springer et al., 1959), the growth stimulation of dietary antibiotics (Lev and Forbes, 1959), experiments on tumorigenesis (Reyniers and Sacksteder, 1959), development of parasitic infections (Bradley et al., 1967), and for a study of the germ-free state per se compared with the conventional animals (Reyniers et al., 1949, 1960).

The germ-free chickens are less clean than conventional chicks due to the high humidity in the units and also by the frequent occurrence of anal blockage and loose nature of the feces of the germ-free chickens. The morphology and function of the gastrointestinal tract are altered in the absence of viable flora in the germ-free animals (Reyniers, 1960). The intestinal mucosa had less lymphatic development and less connective ti mass in germ-free chickens and the general picture was that there were more absorptive elements but fewer and less well developed elements of defense (Reyniers et al., 1960). Gordon (1960) found about 3 times greater numbers of reticulo-endothelial cells in the mucosa and the submucosa of the ileum of young conventional chickens than in germ-free chickens. This difference was also noted for "schollen" or globule leukocytes found within the epithelium and in the number of plasma cells and lymphocytes in the submucosa and lamina propria of the lower

ileum. However, the epithelial cell content was greater in germ-free chicks. The amount of lamina propria in the total area studied was 25.6 ± 0.9% in germ-free chicks and 36.8 ± 1.4% in conventional chicks. Eyssen and DeSomer (1967) reported that the weight of the small intestine was 105% greater in conventional than in germ-free chicks. Enlargement of the cecum was a notable change in several species of germ-free animals including the rat, mouse, rabbit and guinea pig (Wostmann and Bruckner-Kardoss, 1959). However, cecal distention was not observed in germ-free chickens and turkeys (Luckey, 1963). The ceca of the germ-free chickens were found to be significantly shorter in length than those of chickens with a normal bacterial flora (Hegde et al., 1969). From gross observation, the large intestine and cloaca of conventional and germ-free chicks were similar. However, the relative wet weight of large intestine per 100 grams body weight was greater in germ-free Leghorn chickens. The feces of germ-free and conventional chicks were grossly similar, but the germ-free chicks were more susceptible to diarrhea. The lymphatic system is poorly developed in germ-free animals. Using the ileo-cecal tonsils of birds as an index of the lymph node development, generally a great difference was found (Reyniers et al., 1960; Gordon, 1960) between germ-free and conventional chickens. The ileo-cecal tonsils of conventional chickens were of larger

size, full and more turgid while ileo-cecal tonsils of germ-free birds were flabby, pale and inconspicuous. This difference persisted till 5 months of age and from then on the difference became less apparent. The relative weight of the trident at the ileo-cecal valve which contains both the ileo-cecal tonsils was consistently and substantially smaller in germ-free chickens than in conventional birds. The concentration of lymphocytes in the ileo-cecal tonsils of the germ-free birds was from 1/5 to 1/20 of that found in birds harboring live bacteria. Thorbecke (1959) found no plasma cells or secondary nodules in the ileo-cecal-colic junction of germ-free chicks at 2, 4, 8, and 14 weeks. They were found in conventional chicks of all ages and in germ-free chicks at 6 weeks of age. In White Wyandotte Bantam chicks, the bursa of Fabricus of conventional chicks were larger per 100 grams body weight than that of germ-free chicks. In germ-free chickens the spleen was of smaller size (Reyniers et al., 1960) but the general structure, color and consistency of the peripheral and cut surfaces were identical with that seen in conventional groups. Distribution of plasma cells was similar in both the groups but these cells were less often found in the thymus, ileo-cecal junction and follicles of the bursa of the germ-free chicks (Thorbecke, 1959). The growth of germ-free chickens reared on a sterilized purified diet was found to be comparable with control chicks reared on

natural commercial diet, but the growth rate is reported to be faster in germ-free chicks (Forbes and Park, 1959; Forbes et al., 1959). Reyniers et al. (1960) found growth and reproduction to be normal but egg production and hatchability poor in germ-free chicks. The red cell morphology, hemoglobin concentration, hematocrit values, and the expressed blood volume were identical in germ-free and conventional chickens, but leukocytes were 2 to 5 times more numerous in conventional chickens. Circulating lymphocytes were also high enough to state that the presence of living microorganisms and/or their products had an effect on the numbers of lymphocytes (Luckey, 1963). The low level of antibody containing globulin fractions is one of the characteristics of the germ-free animal (Balish and Phillips, 1966; Thorbecke et al., 1957). The gamma-1, gamma-2 and beta fractions of the globulin fraction of the blood of germ-free chicks were also low (Wostmann, 1959) and unlike in conventional chicks, no change in serum gamma globulin occurred in germ-free chicks as they matured. It is not known if the low gamma globulin of the germ-free chicks is "innate" or produced as a result of an unknown antigenic stimulus. Boggs et al. (1967) studying granulocytopoiesis in germ-free mice reported a lower concentration of neutrophils in blood of germ-free mice but the presence or absence of microorganism did not alter the overall granulocytopoiesis.

A more positive oxidation-reduction potential of

cecal contents appears directly related to the absence of gastrointestinal microflora. Balish and Phillips (1966) and Springer (1968) found the oxidation-reduction potentials of bacteria-free cecal contents of the chicken strongly positive while those of the conventional chicken were strongly negative. Balish and Phillips (1966) reported that the pH was higher in all segments of the gut in germ-free chicks when compared to that seen in conventional chicks. The above studies indicated that the germ-free chicks show acceptable normal growth and reproduction. In addition, perosis or spontaneous tumors are not common in germ-free chicks. They survived x-irradiation better than conventional chicks when the dosage was below 800 r at the rate of 32 r per hour (McLaughlin et al., 1958). No gross physiological abnormality has been reported in germ-free chicks. One condition called "jitters" was reported by Gordon et al. (1959) in germ-free chicks due to cellular proliferation in the brain.

The serum of germ-free animals has been shown to have a low globulin content and very few anti-bacterial agglutinins, though complement and heterohemagglutinins are present. This, together with paucity of leukocytes and poor phagocytic response, may make the germ-free animals very susceptible to pathogens, although, surprisingly, germ-free animals were found to be very effective in clearing injected particles or dead bacteria (Luckey, 1963). The pathology of many infectious diseases has been studied in germ-free animals.

Pathogenicity of Intestinal Parasites and Parasitism in Gnotobiotic Hosts

Several investigators have utilized gnotobiotic hosts for the study of certain aspects of the host-parasite relationship. In contrast to the research on axenic (in vitro) cultivation of parasitic organisms which has been directed towards learning the biochemical and immunochemical characteristics of the organisms, the use of gnotobiotic hosts has been oriented towards the in vivo study of the etiology and pathogenesis of certain diseases. A number of studies on the development and pathogenesis of intestinal protozoa and helminths of human and animal importance have been done in gnotobiotic hosts. These studies have shown the relationship between the host, the parasite and the host's intestinal microflora (chiefly bacteria and fungi). Phillips et al. (1955) showed that Entamoeba histolytica can produce pathological lesions in guinea pigs only when species of bacteria such as Escherichia coli and Aerobacter aerogenes are present. However, another parasitic protozoan, Pentatrichomonas (Trichomonas) hominis, developed in large numbers in germ-free guinea pigs (Phillips, 1962). Pentatrichomonas (Trichomonas) vaginalis, when subcutaneously injected into germ-free guinea pigs, produced large lesions, but similar administration in conventional guinea pigs resulted in disappearance of the protozoans in a few days (Newton et al., 1960).

Experimental infection of gnotobiotic mice with Nematospiroides dubius and Nippostrongylus brasiliensis

have been produced and studied (Newton et al., 1959; Westcott, 1971). In general, more parasites developed, infections were of longer duration, and more helminth eggs were produced in the conventional than in germ-free hosts. Eosinophilia was marked in germ-free mice following nematode infection, but no eosinophilia was seen in conventional mice. Nodule development in the intestinal wall was seen in both types of hosts, however, the nodules disappeared from conventional hosts rapidly but persisted up to 60 days in germ-free mice. Weinstein et al. (1962) reported that the larvae of N. dubius will not develop to the infective stage in feces from germ-free mice as they normally do in feces of conventional mice. At least in this case, the intestinal microflora contributed to the prolonged survival and egg production of the helminth species. Another interesting study by Newton et al. (1959) revealed that the mouse helminths N. dubius and Hymenolepis nana, which do not develop to maturity in the conventional guinea pigs, can do so in the germ-free animal.

Johnson et al. (1967) and Rohovsky and Griesemer (1967) found feline infectious enteritis in the germ-free cat is a mild, nonfatal disease with symptoms of leukopenia, thymic atrophy and lymphoid depletion, but without morphologic intestinal lesions and clinical signs. In SPF cats, clinical signs, ultrastructural alteration of the intestinal mucosa and reduced enzyme activity were noted (Johnson et al., 1967; Fowler and Rohovsky, 1970).

Using bacteria-free turkeys, Doll and Franker (1963) and Franker and Doll (1964) indicated that host's flora may affect the course of infection with the nematode H. gallinarum or the protozoan H. meleagridis. Subsequently Bradley et al. (1964) and Bradley and Reid (1966) demonstrated a dual etiology involving a protozoan (H. meleagridis) and a single species of bacteria (E. coli, C. perfringens or B. subtlis) for infectious enterohepatitis in turkeys. Springer et al. (1970) reported that the bacterial requirements for producing infectious enterohepatitis in bacteria-free chickens were different from those for the disease in bacteria-free turkeys. They attributed the role of bacteria in the pathogenesis of infectious enterohepatitis as to make the cecal environment suitable for the survival of H. gallinarum since histomonads have been found to survive in bacteria-free hosts. An enhancement of virulence cannot be overruled in this infection as in the case of human amebiasis. In his early studies on the ameba-bacteria relationship Phillips (1964) stated that it was almost certain that the bacterial flora acted by "providing a suitable environment, physical and chemical, for excystation and establishment of lumen infection until such time as the ameba enter the tissue." Recently, Phillips and Gorstein (1966) have demonstrated that various bacterial species alter the virulence of E. histolytica grown in ameba-trypanosome cultures, as

measured by subsequent inoculation into animals. Wittner and Rosenbaum (1970) studying the role of bacteria in modifying the virulence of E. histolytica found that the increased virulence is associated only with contact of ameba with live bacteria and speculated transfer of an episome-like virulence factor from bacteria to the protozoans.

Reid and Botero (1967) reported the growth of the cestode, Raillietina cesticillus, in bacteria-free chickens and concluded that no contribution to the establishment of the tapeworm or interference from the normal bacterial flora of the digestive tract occurs. Johnson (1971) reported the growth and development of Ascaridia galli in gnotobiotic chickens and the data indicate an inhibi[illegible] of development of the nematode in the bacteria-free chickens.

On the other hand, Balish and Phillips (1966) reported that oral challenge with Candida albicans resulted in crop infection in all bacteria-free chicks but no infection occurred in conventional chicks. Layton and Simkins (1971), in their studies with Mycoplasma gallisepticum, found higher mortality (63%) in germ-free chicks than in conventional chicks (38%). In gnotobiotic swine, Kohler and Cross (1969, 1971) have described diarrheagenic effects due to heat-stable filtrates of broth cultures and whole cell lysates of Escherichia coli and Meyer et al. (1964, 1967, 1971) described a polyserositis-like syndrome

due to E. coli in germ-free pigs. These studies all indicate that normal flora has either a beneficial or antagonistic action on many of these pathogenic organisms. Another intriguing role for the associated microflora in the host-parasite relationship, perhaps in determining host specificity, was the resistance of the conventional guinea pigs to Trypanosoma cruzi. However, a majority of the germ-free guinea pigs harbored the trypanasomes in their blood following intracecal inoculation (Phillips and Wolfe, 1959). In contrast, the bacterial flora had no role in the establishment and pathogenesis of E. brunetti in the chicken intestine (Hegde et al., 1969). Clark et al. (1962) also found very little difference in the pathogenicity of E. tenella in conventional and bacteria-free chickens although there was a delay of 12 to 15 hours in the appearance of the 2nd generation merozoites in the feces of gnotobiotic hosts. In a more recent study, Visco and Burns (1966, quoted by Hegde et al., 1969) reported no mortality in 41 gnotobiotic chickens infected with E. tenella as compared to 77% mortality in infected conventional chickens. They concluded that a close relationship exists between the host flora and the protozoan in the production of cecal coccidiosis syndrome. Kemp et al. (1971) reported a delayed development of endogenous stages of E. tenella in germ-free chicks, especially 2nd generation schizonts, gametocytes and oocysts. There was a striking lack of

reticulo-endothelial cells in the lamina propria and submucosa and substantially low numbers of mononuclear inflammatory cells. Thus, the effects of the bacterial flora on the pathogenicity of this species remain unresolved.

NORMAL MICROBIAL FLORA OF CHICKENS

The importance of intestinal microflora to the welfare of the host has been recognized early in the history of microbiology. As a result, the nature of the intestinal microflora of many animal species is well documented in the literature (Smith and Crabb, 1961; Willingale and Briggs, 1955; Dubos and Schaedler, 1962; Smith and Jones, 1963; Savage and Dubos, 1968; Ogata and Morishita, 1969; Savage et al., 1968, 1970; Rall et al., 1970). In avian species, the microflora studies have been limited to turkeys and chickens.

Cook et al. (1954) studied the effects of antibiotics on the intestinal microflora of turkey poults Naqi et al. (1970a, 1970b) studied the intestinal microflora of normal healthy turkeys from 1 day to 8 weeks of age and also in those infected with "blueconb." These studies indicated that in turkey poults shortly after hatching, the intestinal tract is invaded by several species of bacteria. The microorganisms then multiply rapidly reaching high numbers within the first 24 to 48 hours of life. Findings have been similar in other animal species (Lev and Briggs, 1954; Dubos et al., 1965; Smith, 1965b). A number of workers have studied the

normal bacterial flora of conventional chickens (Johansson et al., 1948; Shapiro and Sarles, 1949; Lev and Briggs, 1956; Lev et al., 1957; Huhtanen and Pensack, 1965; Smith, 1965a; Timms, 1968; Barnes and Impey, 1968). Factors such as age, alimentary tract structure and function, diet, feeding habits and environmental factors have been shown to influence the bacterial flora of the normal gut (Johansson et al., 1948; Smith, 1961; Smith, 1965a, 1965b; Smith and Crabb, 1961). All these studies indicated that the numbers of bacteria of all groups were found to be highest in the cecal contents and progressively lower numbers in the posterior large and anterior small intestine, respectively. The organisms constituting the major part of the flora were E. coli, enterococci (Streptococcus fecalis), Lactobacillus sp., Bacteroides sp. and C. perfringens. The absence of Bacteroides sp. in the small intestine, the preponderance of Bacteroides sp. and Lactobacillus sp. in the ceca, and the low levels of C. perfringens in all sites were of particular interest to the investigators. Shapiro and Sarles (1949) found the count of aerobic and anaerobic bacteria to be similar in chickens of different ages. On the contrary, Huhtanen and Pensack (1965) found a preponderance of anaerobes after 2 weeks of age. Their results also indicated that in day-old unfed chicks the flora consisted mainly of S. fecalis. These enterococci gradually disappeared from the duodenum after 6 days of age. The cecum also showed an initially high

count of enterococci and aerobic bacteria. These were replaced by anaerobic types at around 14 days of age.

The normal bacterial flora has been reported to influence the host-specificity of some parasites (Newton et al., 1959). Another interesting phenomenon is the decrease and/or increase in the population of some members of the flora during certain pathological conditions. Balish and Phillips (1966) reported that in C. albicans infection of the crop, the count of enterococci was increased. Naqi et al. (1970a) reported significant differences in the intestinal microflora in turkeys inoculated with an infectious enteritic ("blueconb") agent and uninfected control turkeys. The changes were characterized by a rise in total microbial count of the entire intes[illegible] a significant increase in number of coliforms, lactose nonfermenters and clostridia. Lactobacillus sp. decreased with severe infectious enteritis but increased when the disease was mild. Microflora changes similar to these findings have been observed by Smith and Jones (1963, 1967) and Ogata and Morishita (1969) in pigs inoculated experimentally with an enteric pathogen. Johansson and Sarles (1948) noted that in E. tenella infection, a stimulation of the growth of C. [illegible] occurred with concurrent decrease of Lactobacillus sp. and a rise in blood glucose level during the 5th to 7th day post-infection. This may be related to an i[illegible]terference in glucose metabolism

and a role for the flora in the pathogenesis of cecal coccidiosis. Thus, in cecal coccidiosis the problem to be studied is whether or not the bacterial flora present in the intestine aid or hinder the initiation of the disease and subsequent development of pathological changes. A particular species or a combination of species may (or may not) help excystation of oocysts, subsequent liberation and survival of sporozoites and invasion of cecal epithelium, development of schizonts and/or gametocytes and thereby contribute to the tissue damage.

MATERIALS AND METHODS

I. Production of Gnotobiotic Chickens

Gnotobiotic (bacteria-, fungi- and PPLO-free) chickens were raised in flexible plastic film isolators following the method of Bradley et al. (1967). All isolators, accessories and supplies were obtained from the same source.[1] The methods employed in sterilization, maintenance, and operation of gnotobiotic environment chambers and equipment, the sterilization of feed supplies and the scheme used for determining the microbiological status were the same as those described by Bradley et al. (1967).

Day-old or 19-day-old embryonated White Leghorn chicken eggs were obtained from a commercial hatchery[2] free of Salmonella and Mycoplasma infection and incubated at the laboratory. Prior to introduction into the isolator chambers, all eggs were candled at least twice to insure the viability of the embryos. The surface of the egg shells was sterilized by immersing the eggs (packed in a tubular nylon net) for 8 minutes in a 2% solution of

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[1]G. F. Supply Division, 431 North Quentin Road, Palatine, Illinois 60067.

[2]Florida State Hatcheries, Gainesville, Florida 32601.

mercuric chloride held at 37° C. The eggs were then drawn into the presterilized isolator by means of an egg chute and placed in a plastic tray containing a cotton towel. After removal of the egg chute and sealing of the entry port, the entire isolator was placed in a room held at 37° C and 80-85% relative humidity for hatching. After hatching, the egg shells were removed and the chickens transferred to a plastic wire-floored basket inside the isolator. Sterile feed and water were provided ad libitum.

II. Production of SPF Chickens

Chickens hatching from fertile eggs obtained from the same source as that from which eggs were obtained for production of gnotobiotic chickens were immediately transferred to modified Horsfall-Bauer units. Altogether 10 such units were kept in a room adjoining those in which the plastic film isolators were kept for the production of gnotobiotic chickens. Air entering the Horsfall-Bauer units was sterilized by passage through sterilized fiberglass filter media. Sterile water was supplied in gallon-sized bottles attached to the inlet tube of the unit and the level controlled by gravity flow. Feed consisted of chicken starter mash free of any antibiotics or added chemicals and was of composition meeting National Research Council (NRC) standards. The feed was pasteurized in a hot air oven at 150° C for at least 60 minutes and was supplied to the chickens in a metal self-feeder inside

each unit. Temperature was controlled electrically and ventilation was fan-forced, negative pressure. Before and after each use, the units were cleaned and scrubbed with hot detergent solution and steam sterilized. As far as possible, the units were opened only 3 times during an experiment--to enter newly-hatched chicks, for exposure of the chicks to E. tenella oocysts, and to remove dead birds. Periodically, clinical laboratory tests were conducted to check the pathogen-free nature of the birds. All experimental chickens raised in these units were monitored by standard laboratory methods for the following specific pathogens:

1. the 9 species of Eimeria causing coccidiosis in chickens;
2. the common intestinal helminths of chickens (Ascaridia, Heterakis, and Baillietina species);
3. Salmonella and Pasteurella species; and
4. H. meleagridis.

III. Production of Conventional Chickens

Newly-hatched chickens were transferred from the incubator and reared in electrically-heated battery brooders. Unsterilized chicken starter mash, with no antibiotics or added chemicals, satisfying NRC requirements, and water were made available ad libitum. Before and after each use the pens were cleaned, scrubbed with hot detergent solution, and steam sterilized.

IV. Source of Eimeria tenella

The same strain of E. tenella isolated from a natural case of cecal coccidiosis was used throughout this series of experiments. Oocysts were produced according to need in disease-free 3-week-old conventional chickens. Fecal material from donor chickens was collected at 7-9 days after inoculation with a sublethal dose of oocysts and the fecal debris and other gross particles removed by sieving through 30 and 80 mesh sieves. The oocysts were then sedimented by centrifugation and allowed to sporulate in 2% potassium dichromate solution at room temperature and after sporulation were stored at 5° C. Immediately before use in experimental trials, all oocysts were surface-sterilized with a 0.5% solution of peracetic acid (Doll et al., 1963). Sterility was tested using standard bacteriological and mycological proccdures. Approximately 100,000 sporulated oocysts were administered orally for experimental infection of chickens, using a small syringe with an attached cannula to insure deposition into the crop.

V. Pathological Examinations

Chickens inoculated with oocysts were kept under close observation. Bleeding or any other clinical signs were noted. Blood samples for packed cell volume determination and serum analysis were taken both before

inoculation and on the 7th day after exposure. Bacterial isolates were made on both the control and experimental chickens at necropsy, according to the bacteriological procedure described below. Chickens dying after exposure to oocysts and all those surviving on the 7th day after exposure were necropsied, examined for gross lesions of cecal coccidiosis and the cecal contents examined for various developmental stages of E. tenella. The infection produced in each inoculated group was compared and graded for macroscopic lesions. For histopathological examination, cecal tissues showing gross lesions and cecal tissues from comparable sites from chickens showing no gross lesions were fixed in neutral 10% formalin and sectioned at 5 or 10 μ thickness and stained with hematoxylin-eosin. The system of macroscopic grading of lesions and microscopic grading of parasitism was modeled after Horton-Smith et al. (1961) as follows:

Scheme of Grading of Lesions and Parasitism

Extent of Lesions and Parasitism	Macroscopic Grading of Lesions	Microscopic Grading of Parasitism
0	No detectable lesions	No coccidial stages found even on careful search
+	Small number of lesions	Small number of gametocytes found by careful search (5 or less)

Extent of Lesions and Parasitism	Macroscopic Grading of Lesions	Microscopic Grading of Parasitism
++	Moderate number of lesions with some hemorrhage	Small number of 2nd generation schizonts and/or gametocytes in scattered groups with some associated tissue damage
+++	Numerous lesions and hemorrhage	Numerous widely distributed gametocytes in localized foci with appreciable tissue damage
++++	Numerous lesions with severe hemorrhage and cecal enlargement	Numerous schizonts and/or gametocytes with widespread tissue damage

VI. Bacteriological Procedures

A. Determination of microbial flora of the cecum in conventional chickens

The development of cecal microbial flora in disease-free young conventional chicks and those inoculated with 100,000 sporulated oocysts was studied as follows. Newly-hatched White Leghorn chicks were raised on standard electrically-heated battery brooders and fed unsterilized feed and water *ad libitum*. Four uninfected control and 4 infected chickens were necropsied at various age intervals: day-old (control only), 6-day-old, 1, 2, 3, 4 and 5 weeks of age. Inoculation with oocysts was adjusted so that the day of necropsy would be 7 days after exposure. At necropsy, the viscera was exposed and the cecal pouches together with 2 inches of anterior small intestine and

posterior large intestine were transferred to a sterile petri dish. Using aseptic procedures, the cecal pouches were opened, the sterile tip (0.0025 cm^2) of an inoculating needle was inserted into the cecal contents, withdrawn, and the material on the loop streaked on the surface of each of the various solid media used in a standard petri dish or inoculated into a tube of broth or semisolid medi . In case of dry cecal contents or a formed cecal core, a drop of sterile pH 7.0, 0.067 M phosphate buffer was used to soften the material prior to insertion of the sampling loop. Aerobes were enumerated on Difco[R1] brain heart infusion agar; enterococci in Difco[R] azide-dextrose broth supplemented with 10 p.p.m. methylene blue and 1.5% agar; and coliforms on MacConkey agar. The medium used for determining the anaerobic populations was Difco[R] brain heart infusion agar supplemented with 0.1% cysteine-hydrochloride and 0.1% bovine serum. C. perfringens was enumerated on Difco[R] sulphite polymyxin sulphadiazine agar. For fungus isolation, Difco[R] Sabouraud dextrose agar and Difco[R] Pagano-Levine agar with antibiotics were used; for protozoan isolation, Difco[R] Balamuth medium was used. Time of incubation was 18 hours for coliforms, 24 hours for clostridia and 48 hours for all other organisms. After incubation, the colonies from each of the 4 uninfected controls and the 4 infected ceca were counted and mean numbers calculated.

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[1]Difco Laboratories, Detroit, Michigan 48232.

B. Determination of microbial flora of the cecum in germ-free, SPF, and conventional chickens

At necropsy, the primary isolation media used for the recovery and identification of bacteria, fungi and PPLO from the ceca of the germ-free, SPF and conventional chickens are listed in Table I. The criteria listed by Breed et al. (1957) were employed for characterization, identification, and verification of bacterial species. Anaerobic plates were incubated in a Gaspack[R1] Anaerobic Jar #60410 using Gaspack[R] gas generator envelopes for generating hydrogen and carbon dioxide gas. Methylene blue was used as the indicator.

VII. Packed Cell Volume

Hematocrit values were determined by drawing the blood into a heparinized microcapillary tube and centrifuging the blood in an International Microcapillary Centrifuge[2] for 5 minutes at 15,000 r.p.m. The packed cell volume (PCV) was directly read using an International Capillary Reader.[2]

VIII. Total Serum Protein

Total serum protein was determined by the method of Weichselbaum (1946) with slight modifications. A standard

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[1]Baltimore Biological Laboratory, Baltimore, Maryland.

[2]International Equipment Company, Needham Heights, Massachusetts.

Table I

Primary Isolation Media Used for the Recovery and Identification of Bacteria and Fungi from Ceca of SPF and Conventional Chickens

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Difco^{R} azide-dextrose broth with 10 p.p.m. methylene blue and 1.5% agar

Difco^{R} blood agar with 0.5% defibrinated bovine blood

Difco^{R} brain heart infusion agar with 0.1% cysteine-Hcl and 0.1% bovine serum

Difco^{R} Brewer's anaerobic agar

Difco^{R} blood agar with 0.5% blood agar and neomycin

Difco^{R}-Columbia broth

Difco^{R} enterococci presumptive broth

Difco^{R} MacConkey agar

Difco^{R} Pagano-Levine base with triphenyl tetrazolium chloride and antibiotics

Difco^{R} PPLO broth with antibiotics

Difco^{R} Sabouraud dextrose agar with antibiotics

Difco^{R} sulphite, polymyxin sulfadiazine agar

Difco^{R} Salmonella-Shigella agar

Difco^{R} thioglycollate medium without dextrose

Difco^{R} tomato juice agar special

Difco^{R} trypticase soy broth

curve was plotted using various dilutions of a 10% bovine serum albumin solution and corresponding optical density in a Turner Spectrophotometer[1] at a wave length of 540 nm. In the procedure, 0.1 ml of unknown serum and 8.0 ml of stable biuret reagent[2] were mixed and incubated at 37° C for 45 minutes. The optical density was then read at 540 nm. The total serum protein was directly calculated from the standard curve and expressed as grams per 100 ml of serum.

IX. Serum Electrophoresis

Electrophoresis of serum proteins was done by Microzone^{R} Electrophoresis[3] on cellulose-acetate membranes using pH 8.6, 0.075 ionic strength barbital buffer. Using the applicator, 0.50 µl of the serum sample was applied onto the membrane and electrophoresised at 300 volts for 35 minutes. The membrane then was stained in Ponceau-5 fixative dye solution for 8 minutes and cleared in 33% cyclohexanone in absolute alcohol for 1 minute. After drying, the membrane was scanned in a Microzone^{R} Densitometer[4] and the chart tracings evaluated to obtain the component percentages.

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[1] G. K. Turner Associates, Palo Alto, California.

[2] Hycel, Inc., Houston, Texas.

[3] Beckman Instruments, Inc., Fullerton, California.

[4] Ibid.

X. Exposure to Bacteria and Fungi

Single species or combinations of bacteria or fungi to be used along with E. tenella for experimental exposure were selected on the basis of microbial isolates from ceca of conventional chickens having very severe coccidiosis. Approximately 0.5 ml of 24 to 48 hour broth culture of the respective organism was administered orally using a cannula. In case of fungi other than Candida sp., 0.5 ml of a heavy spore suspension was administered. C. albicans was grown in Difco^{R} Sabouraud dextrose broth at 37° C for 48 hours and 0.5 ml of the broth was administered orally. Bacterial species were administered 24 hours before E. tenella oocysts were given; fungal suspensions were given 48 hours prior to oocyst administration.

RESULTS

Development of Microbial Flora in the Cecum of Conventional Chickens Inoculated with E. tenella and Uninoculated Controls

The predominant aerobic and anaerobic bacterial species in the cecum at different ages of control chicks and of those exposed to E. tenella oocysts are listed in Table II and Table III.

In day-old and 2-day-old conventional chicks, cecal organisms were predominantly enterococci (S. faecalis) with E. coli making up most of the remainder. In noninfected chicks at 7 days of age, enterococci were not found to be the predominant organisms in the ceca, these being re- by E. coli and Lactobacillus sp. In E. tenella-infected chickens, the enterococci were greatly outnumbered by other species, especially E. coli and Bacteroides sp., but numbers of Lactobacillus sp. were greatly reduced as compared to noninfected controls.

On the 14th day of age in noninfected conventional chicks, E. coli and Lactobacillus sp. were predominant with few enterococci, and Bacteroides sp. were rare. In the infected chicks, E. coli was the most predominant species, and Lactobacillus sp. were not detected.

At 21 and 28 days of age, E. coli and Lactobacillus sp. predominated in the noninfected group while in the infected group, E. coli, Bacteroides sp., enterococci and Lactobacillus sp. were detected. A stimulation of growth of E. coli, Bacteroides sp. and S. fecalis was observed during E. tenella infection while the growth of Lactobacillus sp. was suppressed. At 35 days of age, the same pattern was observed except that in chickens infected with E. tenella, C. perfringens and anaerobic fecal streptococci were also detected.

Fungal isolates were rare. In 6-day-old noninfected chickens, Candida and Mucor species were detected, but in the infected ceca, only Mucor species were seen. At 1, 3 and 5 weeks of age, Candida species were detected in noninfected groups and in 2-week-old infected chickens Mucor species were detected. In 4-week-old chickens, no fungi were detected in either noninfected and infected groups.

Bacterial Isolates in 3-Week-Old SPF and Conventional Chickens Inoculated with E. tenella and Uninoculated Controls

Bacterial isolates in 3-week-old SPF and conventional chickens inoculated with E. tenella were more or less similar and are shown in Tables VIII and IX. C. perfringens was isolated from infected and noninfected SPF chickens and from infected conventional chickens more frequently than from noninfected conventional chickens. A

stimulation of growth of C. perfringens was noted both in infected SPF and conventional chickens (Table X). In noninfected conventional chickens, C. perfringens was isolated in only 2 trials while in infected chickens this species was isolated in all the trials. The number of colonies of C. perfringens developing from a standard inoculum was higher for SPF chickens than for conventional chickens. Occurrence of fungal species was also frequent in SPF chickens, especially in the noninfected ones. Candida species were isolated from noninfected controls in 4 of the 8 trials, while Candida species were isolated from only 1 of the 8 trials in conventional chickens (Tables VIII and IX). Mucor species were infrequent and were isolated only in 1 trial from a noninfected conventional chicken inoculated with E. coli, C. perfringens and S. fecalis.

Pathology due to E. tenella in bacteria-, fungi- and PPLO-free chickens is reported in Table IV. Clinical symptoms like bleeding, anorexia, weakness, and drooping were not noted in any of these chickens. No mortality occurred in 32 of these chickens raised bacteria-, fungi- and PPLO-free. The macroscopic grading of lesions at necropsy in all these cases was negative since there was no visible thickening, hemorrhage, core formation, or sloughing of the mucosa. Cecal enlargement was also not noted. The appearance of ceca, liver, kidneys, small and large intestine, bursa of Fabricus, spleen, heart, muscles,

and lungs were normal as compared to the viscera from noninfected controls raised bacteria-, fungi-, PPLO-free and also those raised specific pathogen-free and conventionally. Histopathologically, there was no tissue damage, hemorrhage, sloughing or thickening of the mucosa evident on microscopic examination. However, E. tenella appeared to survive and undergo some development since endogenous stages were seen in the epithelial cells of the mucosa, especially of the gland fundi. These endogenous stages were identical to immature schizonts and early gametocytes. No large 2nd generation schizonts containing mature merozoites were seen. Cecal coccidiosis as described by Tyzzer (1929) and Tyzzer et al. (1932) was not seen in these chickens when exposed experimentally to a standard inoculum of E. tenella oocysts.

There was no decrease in hematocrit values in bacteria-, fungi- and PPLO-free chickens exposed to E. tenella (Table XI). On the contrary, hematocrit values increased from a mean pre-infection volume of 27 to 28 on the 7th day post-infection.

In chickens harboring only C. albicans, exposure with E. tenella caused mild lesions of thickening but no profound bleeding or sloughing (Table V). Histologically, the tissue damage was negligible but endogenous stages were seen, especially immature schizonts. Denudation of mucosa was minimal. Hematocrit values showed an increase of 1% in these chickens on the 7th day post-infection.

In chickens harboring Mucor species, moderate numbers of lesions with some hemorrhage and thickening was noted macroscopically. Histologically, there was moderate tissue damage and large numbers of endogenous stages including oocysts were seen.

In chickens monocontaminated with anaerobic fecal streptococci and exposed to E. tenella there were no visible lesions or hemorrhage, but histologically, endogenous stages were evident. Tissue damage, sloughing of mucosa, hemorrhage, and large 2nd generation schizonts were also absent. Hematocrit values increased from a pre-exposure volume of 17.0 to a post-exposure volume of 21.5. In chickens monocontaminated with anaerobic fecal streptococci and a suspension of killed E. coli, C. perfringens, S. fecalis, infection with E. tenella did not produce any clinical symptoms, death, visible lesions, hemorrhage or thickening of cecal mucosa. Histologically, no tissue damage, large 2nd generation schizonts, or bleeding was demonstrated, but immature schizonts were seen in the epithelial cells of the mucosa. The hematocrit values were 27 at day of inoculation and 31 on the 7th day post-infection.

In chickens monocontaminated with either Bacteroides sp., C. perfringens, E. coli, or other coliforms like A. aerogenes, moderate numbers of lesions with hemorrhage were noticed (Table VI). Death due to cecal coccidiosis occurred only in 2 out of 12 chickens monocontaminated with S. fecalis, and no mortality occurred in those monocontaminated

with other species of bacteria. In chickens monocontaminated with S. fecalis there was a tendency for the blood to coagulate more rapidly. Partially coagulated blood in the ceca was characteristically present in these chickens. Hematocrit values also showed a reduction in chickens monocontaminated with bacteria and infected with E. tenella (Table XI). Comparatively, chickens monocontaminated either with Bacteroides sp., C. perfringens or S. fecalis developed moderate to severe pathology when exposed to E. tenella. Chickens monocontaminated with E. coli or Lactobacillus sp. developed only mild to moderate lesions, and those monocontaminated with anaerobic fecal streptococci showed little pathology when exposed to E. tenella.

Chickens infected with E. tenella and polycontaminated with 2 or more species of bacteria or fungi showed lesions intermediate between those of monocontaminated and SPF or conventional chickens (Table VII). Heavy mortality (80%) and typical macroscopic and microscopic lesions were noted in chickens polycontaminated with C. perfringens and S. fecalis. Death and typical lesions developed even when day-old chickens polycontaminated with Bacteroides sp., C. perfringens, E. coli and S. fecalis were exposed to E. tenella. Comparatively, pathological manifestations were more severe in chickens polycontaminated either with C. perfringens and S. fecalis, Bacteroides sp. and S. fecalis than in those polycontaminated with C. perfringens and E. coli, Lactobacillus sp. and S. fecalis. Association

of bacteria and fungi favored development of moderate lesions, but did not favor development of greater pathology. However, the pathology was more severe than that seen either with the bacterial species alone or fungus species alone.

Pathological manifestations in E. tenella-infected SPF chickens were typical of cecal coccidiosis (Table VIII). Clinical symptoms like bleeding, anorexia, droopiness and mortality were noted. Thirty-three of the total 88 chickens inoculated died, establishing a mortality rate of 38%. Macroscopic as well as microscopic lesions were observed in all infected chickens establishing an infection rate of 100%. The mean gross lesion score was 2.6 and the mean hematocrit value dropped from a pre-exposure volume of 28.3 to 23.7 on 7th day post-exposure.

In conventional chickens, clinical symptoms were identical with those seen in SPF chickens (Table IX). Infection rate was 100% and mean gross lesion score was 3.1. Twenty-three out of 104 inoculated chickens died due to cecal coccidiosis registering a mortality rate of 22.1%. The mean hematocrit value dropped from a pre-exposure volume of 30.3 to 20.1 on the 7th day post-exposure. The clinical symptoms, gross lesions in the cecum and the presence of large numbers of 2nd generation schizonts, extensive denudation of the cecal mucosa and hemorrhage on histopathologic examination confirmed the presence of cecal coccidiosis as described by Tyzzer (1929) and Tyzzer

et al. (1932). In cases having a gross lesion score of ++++ or +++, endogenous stages were seen extending deep in the muscle layers of the cecal wall and bacteria could often be seen among the damaged mucosal layers. The total serum protein concentration and the serum protein fractions in infected and noninfected conventional, SPF and bacteria-, fungi- and PPLO-free chickens are reported in Table XII. In conventional and SPF chickens infected with E. tenella there was a consistent reduction in total serum protein concentration while such reduction was not seen in bacteria-, fungi- and PPLO-free chickens exposed to E. tenella, when compared to the total serum protein concentration of non-infected controls.

DISCUSSION

The cecal microflora was found to change in chickens 1 to 35 days of age. Also, changes in certain taxonomic groups of bacteria occurred when the chickens were exposed to standard doses of sporulated oocysts of E. tenella. Earlier workers reported changes in the indigenous microflora depending upon the age, feed, and environment of the chickens. Changes in certain groups of bacteria in the ceca have been reported in cases of coccidiosis by Johansson and Sarles (1948) and in cases of "bluecomb" by Naqi et al. (1970a). Enterococci (S. fecalis) was the dominant organism of the ceca of newly-hatched chicks. E. coli was present in small numbers and became dominant only in week-old or older chickens. These results, as well as the occurrence of large numbers of Lactobacillus sp. and E. coli in chickens aged 14 to 35 days old, and the constant isolation of Bacteroides sp. agree with the results obtained by earlier workers in chickens (Huhtanen and Pensack, 1965; Timms, 1968), and in turkeys (Naqi et al., 1970b).

Rall et al. (1970) studied the distribution of bacteria in feces of swine and identified E. coli, other lactose fermenters, lactose nonfermenters, Staphylococcus

sp., Clostridium sp., enterococci and Lactobacillus sp. They concluded that distribution of bacterial cells is nonrandom in feces and organisms might occur as discrete microcolonies rather than as individual cells in samples. Jones and Griffiths (1964) reported that in soil, bacteria occur as colonies and Savage et al. (1968) also reported the occurrence of colonies of bacteria on the walls of the gastrointestinal tract in rodents. Kolacz et al. (1970) using conventional dilution techniques noted more variation among samples than among animals for several groups or organisms. Thus, the concept of bacteria occurring in colonies in fecal samples and in the gastrointestinal tract makes meaningful ecological interpretation of data derived from conventional dilution techniques difficult because such information may constitute absolute density data and not indicate the actual number of colonies present. Gr[illegible] et al. (1966) reported successful isolation of Bacteroide sp. from SPF swine after vigorous swabbing of the mucosa to elute the entrapped bacteria. Since the dilution technique has such definite limitations, the technique of taking samples using a standard loop (0.0025 cm^2) was used in this study. This technique has been successfully employed by Rell et al. (1970).

The very rare occurrence of Clostridium sp. in apparently healthy conventional chickens in this study agrees with the findings of Huhtanen and Ponsack (1965). This rare occurrence of species like C. perfringens which,

though ubiquitous in nature, has been variously explained. Lev et al. (1957) reported that C. welchii (C. perfringens) was present in day-old chicks in "infected" quarters but not in "uninfected" quarters. They established a correlation between the effects of antibiotics on the young bird in the presence of this organism. Huhtanen and Pensack (1965) failed to substantiate this report. Naqi et al. (1970b) found clostridia lactose nonfermenters and Bacteroides sp. present in turkeys early in life, but the organisms decreased in numbers or even were absent in older turkeys. They believed that such organisms, on account of their pathogenic and invasive properties, are eliminated by the defense mechanisms of the host, as reported by Dubos et al. (1965). This contention seems unlikely since Timms (1968) reported regular isolation of Bacteroides sp. and C. welchii (C. perfringens) from ceca of chickens 18 days, 7 weeks, and 5 months old. These species were, however, absent in the small intestine.

In the present study, C. perfringens was isolated from SPF chickens frequently. These SPF chickens were healthy and no "infection" was noticed. They were raised on pasteurized feed and water. Also, Bacteroides sp. were isolated from these SPF chickens as well as from conventional chickens. Moreover, in agreement with the report by Johansson and Sarles (1948), in chickens exposed to E. tenella, a stimulation of growth of C. perfringens occurred

both in SPF and conventional chickens after exposure to E. tenella. In conventional chickens with cecal coccidiosis, C. perfringens was regularly isolated, in contrast to non-exposed controls in which this organism was isolated in only 2 out of 8 trials. In 1 of those trials, the other chicks of the experimental group were deliberately inoculated with this organism. The mechanisms by which the intestinal bacterial population is caused to fluctuate were not ascertained in this study, but factors such as antagonism between bacteria, competition for common nutrients, and changes in pH and oxidation-reduction potential may play an important part (Hentges, 1967, 1969; Lev et al., 1957; Meynell, 1963; Schaedler et al., 1965). The present study confirms the observation of Johansson and Sarles (1948) that growth of C. perfringens in the cecal flora of chicks shows an increase during infection with E. tenella, and growth of Lactobacillus sp. show a reduction. In 7-day-, 14-day-, 21-day-, 28-day- and 35-day-old chickens with cecal coccidiosis, a suppression of growth of Lactobacillus sp. and a stimulation of growth of Bacteroides sp., C. perfringens, and E. coli was noted. The factor(s) responsible for suppression of an anaerobic species during cecal coccidiosis which occurs abundantly in normal, healthy chickens and stimulation of a species which occurs only sparsely in normal chickens is not yet known. It may be that during the prepatent and/or patent period of cecal coccidiosis, factor(s) favoring

growth and multiplication of C. perfringens may be readily available with a concomitant reduction of those favoring growth and multiplication of Lactobacillus sp. A difference in severity of the cecal coccidiosis syndrome was noted between chickens monocontaminated with C. perfringens and chickens monocontaminated with Lactobacillus sp. In the former, the pathological manifestations were more severe than the latter. Also, more severe pathology developed in chickens monocontaminated either with S. fecalis, E. coli or Bacteroides sp. than in those monocontaminated with Lactobacillus sp. when exposed to standard doses of E. tenella oocysts. This indicates that the influence of microbial flora in the pathology of cecal coccidiosis depends upon the species involved. This is much more clearly evident when the pathological manifestation in chickens monocontaminated with anaerobic fecal streptococci and also those monocontaminated with C. albicans is compared with that seen in chickens monocontaminated with other species of microorganisms. Little pathology was noted in chickens harboring only anaerobic fecal streptococci and inoculation of heat-killed E. coli, C. perfringens and S. fecalis did not influence the development of symptoms or pathology.

Pathology and clinical symptoms were negligible in chickens monocontaminated with C. albicans and only mild to moderate lesions developed in chickens monocontaminated with Mucor species. Under natural conditions, fungi seem

to have little influence in the development of cecal coccidiosis. In healthy conventional chickens, fungi occurred only infrequently. This may be due to the antagonism between the fungi and bacteria which is well known in microbiology. Balish and Phillips (1966) found that in the presence of bacteria, C. albicans was able to produce no lesions in the crop of chickens, but in the absence of other microflora, C. albicans produced crop lesions. In noninoculated SPF chickens, C. albicans was more frequent than in noninoculated conventional chickens. Infection with E. tenella did not influence the growth of this fungus and an inhibition of growth was evident since isolation of fungi in chickens having cecal coccidiosis was infrequent, both in SPF and conventional environments.

A comparison of the pathology of cecal coccidiosis in SPF, conventional, and polycontaminated chickens raised in plastic film isolators clearly shows the influence of the environment and microflora in cecal coccidiosis syndrome. The mortality rate from coccidiosis was higher in infected SPF chickens than in infected conventional chickens. The occurrence and the number of colonies of C. perfringens was higher in SPF chickens than in conventional chickens. This was the only substantial difference in the microflora of these two groups. In polycontaminated chickens raised in plastic film isolators (these are then designated as "conventionalized" chickens), the mortality rate was high when C. perfringens and S. fecalis were members of the

microflora. A possible role for these organisms in causing death in chickens cannot be ruled out. Balish and Phillips (1966) reported vascular and renal invasion by enterococci in chickens and Domermuth and Gross (1969) reported acute septicemia of young and bacterial endocarditis of older birds due to S. fecalis. Diarrhea, lesions of hemorrhagic necrotic enteritis and death have been reported in newborn piglets due to C. perfringens type C infection (Field and Gibson, 1955; Barnes and Moon, 1964; Bergeland, 1965).

In conventional chickens the lesions and reduction in hematocrit values as a result of E. tenella infection were more noticeable than in SPF chickens, despite a lower rate of mortality. Johnson and Reid (1970) reported that, in certain cases, lesions will be more severe in live birds than in dead birds during an outbreak of cecal coccidiosis. The failure of chicks to recover from the shock due to the large number of developmental stages within the mucosal layers sufficiently early before cecal bleeding occurs may be influencing the death rate (Bertke, 1963). The role of bacterial species like C. perfringens and S. fecalis in the initiation of this suspected shock or in the failure of chicks to recover from this shock, if any, is not known.

Another feature of cecal coccidiosis, unrestricted hemorrhage with no coagulation, was seldom seen in chickens harboring only S. fecalis. Bacteria may have a definite

role in keeping the blood noncoagulated since polycontamination with C. perfringens and S. fecalis or C. perfringens, E. coli, Bacteroides sp. and S. fecalis in chickens resulted in classical cecal coccidiosis syndrome with hemorrhage beginning about 96 hours post-exposure, and presence of fresh blood and blood-filled cecal contents on the 7th day post-exposure. Mortality also occurred in these groups.

The influence of bacteria as a group and of the various taxonomic groups is more evident when 1 day-old chickens are exposed to E. tenella. Bacteria-, fungi-, and PPLO-free chickens when exposed on the day of hatch did not develop the pathognomonic lesions of coccidiosis due to E. tenella. No mortality occurred in these groups. However, when the inoculation was preceded by inoculation with single or multiple species of bacteria, lesions developed. Mortality and bloody cecal contents were noted only in the group polycontaminated with Bacteroides sp., E. coli, C. perfringens and S. fecalis. Groups monocontaminated with S. fecalis developed mild lesions, but did not show mortality or blood-filled ceca. The ceca characteristically contained only partially clotted blood.

Day-old chickens are generally regarded as less susceptible to E. tenella than chickens 2, 3, or 4 weeks old. Rose (1967b), studying the excystation of E. tenella oocysts, observed rapid and greater excystation in chicks

aged 4, 5 and 6 weeks and less successful excystation in 1 day- or 1 week-old chickens. The greater susceptibility of the 4, 5 and 6 week-old chickens was thus ascribed to the successful rapid excystation of the oocysts and liberation of large numbers of sporozoites in the cecal lumen. The low susceptibility of day-old or week-old chickens was likewise ascribed to the less successful excystation of oocysts as a consequence of the less powerful grinding action of the immature gizzard and dearth of sufficient trypsin in the newly-hatched or young chickens. Trypsin, bile, and hydrogen-ion concentration are some of the factors contributing to the excystation (Levine, 1942; Ikeda, 1955, 1956, 1960; Hibbert et al., 1969). Hibbert et al. (1969) found that oocysts of many species of Eimeria, including E. tenella, failed to excyst if bile alone or trypsin alone is present. They also found that oocysts of E. bovis and E. ellipsoidalis readily excyst in bovine bile containing a heavy suspension of bacteria and fungi. The specific action of the bile or the bacteria and fungi in the excystation is not known.

A structural alteration of the walls of the sporulated oocysts expelled in the feces of exposed chickens was observed by Lotze and Leek (1969). It is possible that enzymes like trypsin can enter readily into the oocysts whose wall is structurally changed. The present study indicated that development of cecal coccidiosis as

a result of rapid and greater rate of excystation seems to occur in day-old chickens harboring certain bacterial species which are also regularly isolated from ceca of chickens showing typical lesions of cecal coccidiosis while excystation and development of the disease seems to be less successful in day-old chickens monocontaminated with S. fecalis. In chickens 3 weeks old, less successful and slow excystation seemed to have occurred when only S. fecalis or E. coli or both was harbored in the ceca, if the rate and degree of excystation is correlated with the development of the disease. In the newly-hatched conventional chicken the only microorganisms predominant were S. fecalis and E. coli. It was also found that the pathological manifestations of coccidiosis were greater if C. perfringens was associated with either S. fecalis or E. coli. Whether C. perfringens aids in the excystation of the oocysts is not known and has not been studied.

Normal excystation and subsequent liberation of sporozoites may not be the sole process which will influence the development of pathological manifestations in E. tenella infection. Horton-Smith et al. (1963) reported normal excystation of oocysts and penetration of liberated sporozoites in immune chickens. It is also known that much of the pathology in cecal coccidiosis is due to the maturation of enormous numbers of the large 2nd generation schizonts which destroy the epithelial cells,

causing sloughing of the mucosa and hemorrhage. The role of bacteria and/or fungi in favoring the rapid development of the endogenous stages, if any, is not known.

The present study shows that typical cecal coccidiosis originally described by Tyzzer (1929) and Tyzzer et al. (1932) failed to develop in chickens raised bacteria-, fungi- and PPLO-free. No clinical symptoms, mortality or decrease in hematocrit values following exposure with standard doses of sporulated oocysts were observed. Likewise, no visible lesions were noted in these chickens. Chickens raised simultaneously under SPF and conventional environments and inoculated with the same standard dose of sporulated oocysts exhibited the classical cecal coccidiosis syndrome.

There was also a marked difference between bacteria-free and SPF or conventional chickens based on histopathologic findings. Ceca of the SPF and conventional chicks inoculated with E. tenella oocysts and necropsied on the 7th day post-exposure showed extensive denudation of the mucosa, hemorrhage and large numbers of 2nd generation schizonts. In severe cases, the hemorrhage and tissue damage even reached the muscular layers, and the entire mucosal layer was seen to be stripped down to the base of the submucosa. Large 2nd generation schizonts lying mostly free and a few within their host cells were the predominant endogenous stage seen in such cases. But in the ceca of

bacteria-, fungi- and PPLO-free chickens exposed to E. tenella oocysts and necropsied on 7th day post-exposure, no extensive denudation of mucosa, hemorrhage or large 2nd generation schizonts were seen. The mucosal layer was more or less intact and other tissues were undamaged; no large 2nd generation schizonts were seen. A few endogenous stages identified as immature schizonts were noticed in the epithelial cells of the mucosa, especially those lining the gland fundi. This location of the stages suggests that they may be the 1st generation schizonts developing very slowly. This suggests that in bacteria-, fungi- and PPLO-free chickens the degree and rate of development of endogenous stages are markedly inhibited. This inhibition of the development of endogenous stages, especially the pathogenic and immunogenic 2nd generation schizont may afford protection to the host and the pathological manifestations are not developed. In fact, the bacteria-, fungi- and PPLO-free chickens seem to behave as an immune host. Excystation has occurred in these hosts since endogenous stages are seen in the host cells post-exposure to E. tenella, but their subsequent development seems to have been retarded. Clark et al. (1962), in their studies with bacteria-free chickens, observed a 12-15 hour delay in the appearance of 2nd generation merozoites in the feces of bacteria-free chickens. However, they found no difference in the course of infection in

bacteria-free and conventional chickens. The exact experimental procedures and results are not completely known to compare their results and those from the present study.

Wagenbach et al. (1966), describing a method for sterilizing coccidian oocysts employing Clorox and sulfuric acid-dichromate solution, indicated that they could produce cecal coccidiosis in "gnotobiotic chickens." Since the exact nature of their "gnotobiotic chickens" has not been indicated nor has the description of the experimental procedure and results of their work been given, it is not possible to compare their results with the present work. Their work was designed to obtain sterile oocysts for cultivation of the parasite in tissue culture.

Nyberg and Knapp (1970a, 1970b), examining the oocysts of E. tenella by means of a scanning electron microscope, reported certain structural alterations of oocysts. Monné and Hönig (1954) reported that when oocysts are treated with sulfuric acid prior to sodium hypochlorite, the outer layer of the oocyst wall became wrinkled and apparently elevated away from the inner layer. Employment of such structurally altered oocysts might influence pathogenesis and development of disease processes of coccidiosis. In contrast, the use of 0.5% peracetic acid for surface sterilization of invertebrate

eggs and oocysts has been found to be very effective (Doll et al., 1963; Bradley et al., 1964; Hegde et al., 1969; Springer et al., 1970). Under the light microscope, no damage or changes to the oocyst wall were seen when peracetic acid was used for surface sterilization and therefore it is assumed that peracetic acid is not injurious to the oocyst wall when used according to the method of Doll et al. (1963) and this method was used during the present study. The data obtained in the present study can be compared to those obtained by Visco and Burns (1966, quoted by Hegde et al., 1969) who observed no mortality in bacteria-free chicks when exposed to E. tenella oocysts and by Kemp et al. (1971) who reported a delaying of the development of the endogenous stages in germ-free chicks. In the present study, there was also a delay in the development of endogenous stages observed and this delay may be beneficial, allowing the host to develop sufficient resistance and thereby inhibit the development of clinical symptoms and other manifestations of the disease. It is also possible that the development of the large 2nd generation schizonts, if delayed or inhibited by the absence of microflora, will lead to minimal development of pathology. Inhibition of development and development of fewer numbers of nematode parasites have been found in germ-free hosts including the chicken (Newton et al., 1959 Johnson, 1971). Springer et al. (1970) reported survival

of H. meleagridis without disease production in gnotobiotic hosts. Confirming the synergistic role of certain bacterial species (especially C. perfringens) and H. meleagridis in the production of infectious enterohepatitis in turkeys reported by Bradley et al. (1964), Springer et al. (1970) established that the role of bacteria is more vital for the survival of H. gallinarum larvae than H. meleagridis in the pathogenesis of infectious enterohepatitis.

In E. tenella infections, the exact role of the bacterial species is not known, but the present study indicates that for the disease syndrome to develop in full, certain bacterial species are essential since no mortality or gross lesions were seen in hosts free of any detectable organisms. Johnson et al. (1967) and Rohovsky and Griesemer (1967) found feline infectious enteritis in the germ-free cat to be a mild, nonfatal disease consisting of leukopenia, thymic atrophy and lymphoid depletion but without morphologic intestinal lesions. On the contrary, SPF cats developed clinical signs of typical intestinal involvement as seen in frank infectious enteritis. Since the ceca of the chickens contain the major portion of the gastrointestinal flora (Timms, 1968), E. tenella may have developed an apparent obligate relationship with certain members of this microflora for pathogenesis to commence and this relationship would have influenced the overall host-parasite relationship.

The contribution of indigenous bacteria of the cecal pouches in producing cecal coccidiosis syndrome was first suspected by Ott (1937). Mann (1947) reported the bacteriology of cecal coccidiosis to be similar to that of "six-day disease" incited by C. perfringens, enterococci and coliforms, and characterized by occlusion of ceca with a core and hemorrhage. Johansson and Sarles (1948) also reported a possible involvement of E. coli in the etiology of cecal coccidiosis. Their study and the present study showed that coliforms are present in large numbers throughout the course of cecal coccidiosis. They assumed that the reduced severity in older chickens is due to low coliform count in the cecum. The reduced severity of cecal coccidiosis se in day-old chickens may also be due to the same reason.

SUMMARY AND CONCLUSIONS

Typical cecal coccidiosis did not develop in bacteria-, fungi- and PPLO-free chickens inoculated with a standard dose of infective oocysts of E. tenella. However, small numbers of E. tenella were able to develop at a slower rate in bacteria-, fungi- and PPLO-free chickens as compared with the rate seen in conventional chickens. In chickens monocontaminated with C. perfringens, S. fecalis, E. coli, Bacteroides sp., Lactobacillus sp., Mucor sp. or C. albicans, exposure to E. tenella oocysts resulted in mild cecal coccidiosis. Cecal coccidiosis in chickens polycontaminated with bacteria was comparable with that seen in conventional or SPF chickens. Higher mortality but lower mean gross lesions due to coccidiosis were seen in SPF chickens exposed to E. tenella, compared to that in conventional chickens.

Indigenous flora of the ceca of chickens of various ages was not identical. In very young chickens (up to 1 week of age), enterococci predominated in the ceca while in older chickens (4 to 5 weeks of age), Lactobacillus sp. predominated. Bacteroides sp., E. coli, Lactobacillus sp. and S. fecalis were regularly isolated from conventional chickens at 1, 2, 3, 4 and 5 weeks of age. Isolation of

C. perfringens was infrequent in conventional chickens, but frequent in SPF chickens. Inoculation of chickens with E. tenella resulted in stimulation of growth of C. perfringens both in conventional and SPF birds. Also, large numbers of E. coli and Bacteroides sp., but low numbers of Lactobacillus sp. were seen in the ceca of infected chickens. The data indicate that the indigenous bacteria aid in rapid development of endogenous stages of E. tenella and production of typical cecal coccidiosis.

APPENDICES

APPENDIX A

Table II

Cecal Flora of Young Conventional Chickens Exposed to *Eimeria tenella*

Age in Days	Medium	Number of Colonies per Standard Loop (0.0025 cm^2) from Ceca (mean)	
		Exposed to *E. tenella*	Unexposed Controls
1	Aerobic	N.E.[a]	4,089
	Anaerobic	N.E.	1,755
	Enterococcus	N.E.	2,306
7	Aerobic	482	1,350
	Anaerobic	112	275
	Enterococcus	32	780
14	Aerobic	465	166
	Anaerobic	403	201
	Enterococcus	1	20
21	Aerobic	189	133
	Anaerobic	106	395
	Enterococcus	37	0
28	Aerobic	654	114
	Anaerobic	451	134
	Enterococcus	16	14
35	Aerobic	614	65
	Anaerobic	382	482
	Enterococcus	0	4

[a]N.E.--not exposed.

Table III

Predominant Bacterial and Fungal Species from Chickens Showing Lesions of Cecal Coccidiosis and from Nonexposed Conventional Chickens

	Exposed to Eimeria tenella		Unexposed Controls	
Age in Days	Species Isolated	Number of Colonies per Standard Loop	Species Isolated	Number of Colonies per Standard Loop
1	N.E.[a]	N.E.	S. fecalis	2,306
			E. coli	1,783
7	E. coli	281	E. coli	422
	S. fecalis	32	S. fecalis	780
	Bacteroides sp.	N.C.[b]	Bacteroides sp.	N.C.
			Lactobacillus sp.	570
			Anaerobic fecal streptococci	N.C.
14	E. coli	393	E. coli	146
	Bacteroides sp.	N.C.	Lactobacillus sp.	157
	C. perfringens	N.C.	Bacteroides sp.	N.C.
	S. fecalis	1	S. fecalis	20
21	E. coli	283	Lactobacillus sp.	395
	Lactobacillus sp.	106	E. coli	133
	Bacteroides sp.	N.C.		
	S. fecalis	37		

Table III (Continued)

Age in Days	Exposed to Eimeria tenella: Species Isolated	Number of Colonies per Standard Loop	Unexposed Controls: Species Isolated	Number of Colonies per Standard Loop
28	E. coli	1,383	Lactobacillus sp.	134
	Lactobacillus sp.	45	E. coli	69
	S. fecalis	16	S. fecalis	14
	Bacteroides sp.	N.C.		
35	E. coli	876	E. coli	168
	Bacteroides sp.	N.C.	S. fecalis	4
	Anaerobic fecal streptococci	N.C.	Lactobacillus sp.	N.C.
	Lactobacillus sp.	N.C.	Bacteroides sp.	N.C.

[a]N.E.--not exposed.

[b]N.C.--not counted since no pure colonies developed in media used.

Table IV

Pathology Due to *Eimeria tenella* in Bacteria-, Fungi- and Pleuropneumonia-Like Organisms-Free Chickens Raised in Plastic Film Isolators

Trial	Number of Chickens	Exposure Pattern with *E. tenella*	Clinical Signs	Mortality	Macroscopic Grading of Lesions	Microscopic Grading of Parasitism
1	4	++[a]	No clinical signs or bleeding noted	Nil	0	Mucosa intact, no tissue damage seen. Very rare endogenous stages seen on careful examination
2	4	++	No clinical signs or bleeding noted	Nil	0	Mucosa intact, no tissue damage seen. Very rare endogenous stages seen on careful examination
3	4	++	No clinical signs or bleeding noted	Nil	0	Mucosa intact no tissue damage seen. Very rare endogenous stages seen on careful examination

Table IV (Continued)

Trial	Number of Chickens	Exposure Pattern with *E. tenella*	Clinical Signs	Mortality	Macroscopic Grading of Lesions	Microscopic Grading of Parasitism
4	4	++	No clinical signs or bleeding noted	Nil	0	A few developing endogenous stages seen
5	2	++	No clinical signs or bleeding noted	Nil	0	Very few developmental stages seen
6[b]	4	++	No clinical signs or bleeding noted	Nil	0	Endogenous stages resembling immature schizonts seen
7[c]	4	++	No clinical signs or bleeding noted	Nil	0	Very few endogenous stages seen
8[d]	6	++	No clinical signs or bleeding noted	Nil	0	Very few endogenous stages seen

Table IV (Continued)

Trial	Number of Chickens	Exposure Pattern with E. tenella	Clinical Signs	Mortality	Macroscopic Grading of Lesions	Microscopic Grading of Parasitism
9[e]	3	--	No bleeding or clinical signs noted	Nil	0	0

[a]++ indicates oral inoculation of 100,000 surface sterilized oocysts of E. tenella.

[b]One chick in this trial was necropsied at 24 hours post-exposure for histological study. No endogenous stage was seen in this chicken.

[c]One chick each at 48 hours and 72 hours post-exposure was necropsied for histological study.

[d]The chickens in this trial were exposed on the day of hatch.

[e]Unexposed controls. In ceca or other organs no pathological lesions or abnormality were seen.

Table V

Pathology Due to *Eimeria tenella* and a Single Species of Fungus in Bacteria-Free Chickens Raised in Plastic Film Isolators

Trial	Number of Chickens	Exposure Pattern with *E. tenella*	Species of Fungi	Clinical Signs	Mortality	Macroscopic Grading of Lesions	Microscopic Grading of Parasitism
1	4	++[a]	*Candida albicans*	Nil	Nil	±[b]	Tissue damage very mild; immature schizonts seen.
2	2	++	*Mucor* sp.	Nil	Nil	2.0	2.0 Oocysts seen. Large number of endogenous stages seen. Moderate tissue damage seen.

[a] ++ indicates oral inoculation with 100,000 surface sterilized oocysts of *E. tenella*.

[b] ± indicates doubtful cases.

Table 1

Pathology Due to *Eimeria tenella* and a Single Species of Bacteria in Chickens Raised in Plastic Film Isolators

Trial	Number of Chickens	Exposure Pattern with *E. tenella*	Species of Bacteria	Clinical Signs	Mortality	Macroscopic Grading of Lesions (Mean)	Microscopic Grading of Parasitism (Mean)
1	4	++[a]	*S. fecalis*	Mild bleeding noted 96 hours post-exposure, but stopped in about 120 hours post-exposure	2/4	2.5 A partial clotting of blood was noticed	1.75[b]
2	2	++	*S. fecalis*	No clinical signs or bleeding noted	0/2	1.0	1.0[b]
3	6	++	*S. fecalis*	Nil	0/6	0.66	1.0 Many immature schizonts were seen in the gland fundi

Table VI (Continued)

Trial	Number of Chickens	Exposure Pattern with *E. tenella*	Species of Bacteria	Clinical Signs	Mortality	Macroscopic Grading of Lesions (Mean)	Microscopic Grading of Parasitism (Mean)
4	4	++	*C. perfringens*	Nil	0/4	1.75 Blood clots were seen	1.6
5	1	++	*E. coli*	Nil	0/1	1.0	2.0
6	3	++	*E. coli*	Nil	0/3	0.0	Very rare endogenous stage seen. Tissue damage very little.
7	6	++	*Bacteroides* sp.	Nil	0/6	2.0	2.0
8	3	++	*Lactobacillus* sp.	Nil	0/3	1.5	1.0 Large number of immature schizonts seen. No 2nd generation schizonts were seen. Tissue damage mild.

Table VI (Continued)

Trial	Number of Chickens	Exposure Pattern with *E. tenella*	Species of Bacteria	Clinical Signs	Mortality	Macroscopic Grading of Lesions (Mean)	Microscopic Grading of Parasitism (Mean)
9	4	++	*Aerobacter aerogenes*	Nil	0/4	1.5	2.0
10	3	++	Anaerobic fecal streptococci	Nil	0/3	0.0	1.0 Small number of oocysts seen in the cecal contents. Other endogenous stages also seen.
11	3	++	Anaerobic fecal streptococci	Nil	0/3	0.0	1.0 Very few endogenous stages seen but no oocysts were seen.
12	6	++	Anaerobic fecal streptococci plus killed suspension of *S. fecalis*, *C. perfringens*, and *E. coli*	Nil	0/6	0.0	Immature schizonts seen. No oocysts. Tissue damage very little

Table VI (Continued)

Trial	Number of Chickens	Exposure Pattern with *E. tenella*	Species of Bacteria	Clinical Signs	Mortality	Macroscopic Grading of Lesions (Mean)	Microscopic Grading of Parasitism (Mean)
13[c]	3	++	*S. fecalis*	Mild droopiness noted 5-6 days post-exposure	0/3	2.3 Blood coagulated	2.3 Mature schizonts, gametocytes oocysts seen

[a]++ indicates oral inoculation with 100,000 surface sterilized oocysts of *E. tenella*.

[b]In one chicken in which no visible lesion was noticed microscopically only very mild hemorrhage and tissue damage were seen. Immature schizonts were noticed, but no 2nd generation schizonts were seen.

[c]Chickens exposed on the day of hatch.

Table VII

Pathology Due to *Eimeria tenella* in Chickens Raised in Plastic Film Isolators and Polycontaminated with Bacteria and/or Fungi

Trial Number	Number of Chickens	Exposure Pattern with *E. tenella*	Species of Bacteria Fungi	Clinical Signs	Mortality	Macroscopic Grading of Lesions (Mean)	Microscopic Grading of Parasitism	Remarks
1	5	++[a]	*C. perfringens* *S. fecalis*	Mild bleeding 4th day post-exposure noticed	4/5	3.8	3.0	Typical cecal coccidiosis as seen in exposed conventional chickens
2	5	++	*C. perfringens* *E. coli*	Nil	0/5	1.6	3.0	
3[b]	4	++	*E. coli* *S. fecalis* *Bacteroides* sp. *C. perfringens*	Profuse bleeding 4 days post-exposure detected	2/4	2.7	3.0	Typical cecal coccidiosis

Table VII (Continued)

Trial Number	Number of Chickens	Exposure Pattern with *E. tenella*	Species of Bacteria Fungi	Clinical Signs	Mortality	Macroscopic Grading of Lesions (Mean)	Microscopic Grading of Parasitism	Remarks
4	2	++	*Lactobacillus* sp. *S. fecalis*	Nil	0/2	1.5	3.0	
5	10	++	*Lactobacillus* sp. *Mucor* sp.	Nil	0/10	2.0	2.0	Large number of oocysts seen but tissue damage not very severe
6	3	++	*Lactobacillus* sp. *Mucor* sp.	Nil	0/3	± [c]	1.0	Endogenous stages seen, but tissue damage not very severe.
7	4	++	*Clostridium tetanoides* *Aspergillus* sp.	Nil	0/4	2.0	2.0	

Table VII (Continued)

Trial Number	Number of Chickens	Exposure Pattern with E. tenella	Species of Bacteria Fungi	Clinical Signs	Mortality	Macroscopic Grading of Lesions (Mean)	Microscopic Grading of Parasitism	Remarks
8	4	++	Alcaligenes faecalis Mucor sp.	Nil	0/4	2.0	0.0	No oocysts or other stages [illegible]
9	2	--[d]	S. faecalis E. coli	Nil	0/2	0.0	0.0	The ceca and other organs were comparable to normal unexposed SPF or conventional chickens

Table VII (Continued)

Trial Number	Number of Chickens	Exposure Pattern with E. tenella	Species of Bacteria Fungi	Clinical Signs	Mortality	Macroscopic Grading of Lesions (Mean)	Microscopic Grading of Parasitism	Remarks
10	6	--	E. coli S. fecalis C. perfringens Anaerobic fecal streptococci	Nil	0/6	0.0	0.0	The ceca and other organs were comparable to normal unexposed SPF or conventional chickens
11	1	--	E. coli S. fecalis C. perfringens Bacteroides sp.	Nil	0/1	0.0	0.0	
12	3	--	Lactobacillus sp.	Nil	0/3	0.0	0.0	

Table VII (Continued)

Trial Number	Number of Chickens	Exposure Pattern with E. tenella	Species of Bacteria Fungi	Clinical Signs	Mortality	Macroscopic Grading of Lesions (Mean)	Microscopic Grading of Para-sitic-	Remarks
13	1	--	E. coli	Nil	0/1	0.0	0.0	The ceca and other [illegible] comparable to normal unexposed SPF or conventional chickens

[a]++ indicates oral inoculation with 100,000 surface sterilized oocysts of E. tenella.

[b]The chicks in this trial were exposed to E. tenella oocysts on the day of hatch.

[c]± denotes doubtful case.

[d]-- indicates no oocysts of E. tenella were administered.

Table VIII

Pathology Due to *Eimeria tenella* in Specific Pathogen-Free Chickens

Trial Number	Number of Chickens	Exposure Pattern with *E. tenella*	Clinical Signs	Mortality	Macroscopic Grading of Lesions (Mean)	Microscopic Grading of Parasitism (Mean)	Dominant Species of Microflora Isolated
1	6	++[a]	Bleeding commenced 4th day post-exposure	4/6	2.5	3.0	*E. coli* *S. faecalis* *Bacteroides* sp. *C. perfringens*
2	23	++	Bleeding commenced 4th day post-exposure	15/23	3.0	3.0	*S. faecalis* *C. perfringens*
	8	--[b]	Nil	0/8	0.0	0.0	*S. faecalis* *C. perfringens*
3	16	++	Bleeding commenced 4th day post-exposure	2/16	2.75	3.0	No attempt to isolate any organism was made
	8	--	Nil	0/8	0.0	0.0	

Table VIII (Continued)

Trial Number	Number of Chickens	Exposure Pattern with E. tenella	Clinical Signs	Mortality	Macroscopic Grading of Lesions (Mean)	Microscopic Grading of Parasitism (Mean)	Dominant Species of Microflora Isolated
4	22	++	Bleeding commenced on 4th day post-exposure	10/22	2.75	3.0	S. faecalis E. coli A. faecalis
	8	--	Nil	0/8	0.0	0.0	Lactobacillus sp. A. faecalis E. coli S. faecalis
5	8	++	Bleeding commenced on 4th day post-exposure	0/8	2.4	3.0	E. coli S. faecalis Bacteroides sp. C. perfringens
	3	--	Nil	0/3	0.0	0.0	E. coli S. faecalis C. perfringens C. albicans

Table VIII (Continued)

Trial Number	Number of Chickens	Exposure Pattern with E. tenella	Clinical Signs	Mortality	Macroscopic Grading of Lesions (Mean)	Microscopic Grading of Parasitism (Mean)	Dominant Species of Microflora Isolated
6	8	++	Bleeding commenced 4th day post-exposure	1/8	2.25	3.0	E. coli S. fecalis Anaerobic fecal streptococci C. perfringens A. fecalis
	5	--	Nil	0/5	0.0	0.0	E. coli Lactobacillus sp. Anaerobic fecal streptococci Bacteroides sp.
7	9	++	Mild hemorrhage on 4th day post-exposure	0/9	2.0	2.0	E. coli S. fecalis Bacteroides sp. C. perfringens
	4	--	Nil	0/4	0.0	0.0	E. coli S. fecalis Bacteroides sp. C. perfringens C. albicans

Table VIII (Continued)

Trial Number	Number of Chickens	Exposure Pattern with *E. tenella*	Clinical Signs	Mortality	Macroscopic Grading of Lesions (Mean)	Microscopic Grading of Parasitism (Mean)	Dominant Species of Microflora Isolated
8	3	*E. tenella* plus *E. coli*, *S. fecalis* and *C. perfringens*	Bleeding 4th day post-exposure noticed	0/3	2.0	2.0	*E. coli* *S. fecalis* *C. perfringens* *Lactobacillus* sp.
	3	++	Bleeding 4th day post-exposure noticed	0/3	1.66	2.0	*E. coli* *S. fecalis* *C. perfringens* *Lactobacillus* sp.
	2	*E. tenella* plus *E. coli*	Bleeding 4th day post-exposure noticed	1/2	2.50	2.0	*E. coli* *S. fecalis* *C. perfringens* *Lactobacillus* sp.

Table VIII (Continued)

Trial Number	Number of Chickens	Exposure Pattern with *E. tenella*	Clinical Signs	Mortality	Macroscopic Grading of Lesions (Mean)	Microscopic Grading of Parasitism (Mean)	Dominant Species of Microflora Isolated
8	2	*E. tenella* plus *S. fecalis*	Bleeding 4th day post-exposure noticed	0/2	3.0	3.0	*E. coli* *S. fecalis* *C. perfringens* *Lactobacillus* sp. *Bacteroides* sp. *E. multiformis* *C. pseudotetanopicalis*
	2	*E. tenella* plus *Bacteroides* sp.	Mild bleeding 4th day post-exposure noticed	0/2	3.0	3.0	*E. coli* *S. fecalis* *C. perfringens* *Lactobacillus* sp. *Bacteroides* sp. *E. multiformis* *C. pseudotetanopicalis*
	2	*E. tenella* plus *C. perfringens*	Mild bleeding 4th day post-exposure noticed	0/2	3.0	3.0	*E. coli* *S. fecalis* *C. perfringens* *Lactobacillus* sp. *Bacteroides* sp. *C. pseudotetanopicalis*

Table VIII (Continued)

Trial Number	Number of Chickens	Exposure Pattern with *E. tenella*	Clinical Signs	Mortality	Macroscopic Grading of Lesions (Mean)	Microscopic Grading of Parasitism (Mean)	Dominant Species of Microflora Isolated
8	2	Only *E. coli*, *S. fecalis*, *C. perfringens*	Nil	0/2	0.0	0.0	*E. coli* *S. fecalis* *Lactobacillus* sp. [illegible] sp. *C. perfringens*
	2	*E. coli* alone	Nil	0/2	0.0	0.0	*E. coli* *S. fecalis* *Lactobacillus* sp. *Staphylococcus* sp. *C. perfringens* *E. aerogenes*
	3	--	Nil	0/3	0.0	0.0	*E. coli* *S. fecalis* *Lactobacillus* sp. *C. perfringens* *Bacteroides* sp.

[a]++ indicates oral inoculation of 100,000 surface sterilized oocysts of *E. tenella*.

[b]-- indicates no oocysts of *E. tenella* were administered.

Table IX

Pathology Due to *Eimeria tenella* in Conventional Chickens

Trial Number	Number of Chickens	Exposure Pattern with *E. tenella*	Clinical Signs	Mortality	Macroscopic Grading of Lesions (Mean)	Microscopic Grading of Parasitism (Mean)	Dominant Species of Microflora Isolated
1	7	++[a]	Bleeding on 4th day post-exposure commenced; droopiness present	5/7	3.1	4.0	*E. coli* *S. faecalis* *Bacteroides* sp. *C. perfringens*
2	17	++	Bleeding on 4th day post-exposure commenced; droopiness present	5/17	3.1	3.0	*E. coli* *S. faecalis* *Bacteroides* sp. *Lactobacillus* sp. *C. perfringens*
	10	--[b]	Nil	0/10	0.0	0.0	*Lactobacillus* sp. *E. coli* *S. faecalis* *Bacteroides* sp.

Table IX (Continued)

Trial Number	Number of Chickens	Exposure Pattern with E. tenella	Clinical Signs	Mortality	Macroscopic Grading of Lesions (Mean)	Microscopic Grading of Parasitism (Mean)	Dominant Species of Microflora Isolated
3	17	++	Bleeding noticed on 5th, 6th and 7th day post-exposure	1/17	3.0	4.0	No attempt to isolate micro-organisms was made
	8	--	Nil	0/8	0.0	0.0	No attempt to isolate micro-organisms was made
4	23	++	Bleeding commenced 4th day post-exposure	7/23	2.6	3.0	S. faecalis E. coli P. vulgaris
	7	--	Nil	0/7	0.0	0.0	Lactobacillus sp. E. coli S. faecalis Alcaligenes faecalis

Table IX (Continued)

Trial Number	Number of Chickens	Exposure Pattern with *E. tenella*	Clinical Signs	Mortality	Macroscopic Grading of Lesions (Mean)	Microscopic Grading of Parasitism (Mean)	Dominant Species of Microflora Isolated
5	3	++	Mild bleeding noted on 4th day post-exposure	0/3	2.0	2.0	*E. coli* *S. faecalis* *Bacteroides* sp. *C. perfringens* *Mycoplasma* sp.
	3	--	Nil	0/3	0.0	0.0	*E. coli* *S. faecalis* *Bacteroides* sp. *Mycoplasma* sp.
6	11	++	Mild bleeding commenced on 4th day post-exposure	2/11	2.18	3.0	*E. coli* *S. faecalis* *C. perfringens* *Lactobacillus* sp.
	5	--	Nil	0/5		0.0	*Lactobacillus* sp. *E. coli* *S. faecalis* *Bacteroides* sp.

Table IX (Continued)

Trial Number	Number of Chickens	Exposure Pattern with *E. tenella*	Clinical Signs	Mortality	Macroscopic Grading of Lesions (Mean)	Microscopic Grading of Parasitism (Mean)	Dominant Species of Microflora Isolated
7	8	++	Mild bleeding noticed	2/8	3.0	3.0	*E. coli* *S. fecalis* *C. perfringens* *Proteus* sp. *Lactobacillus* sp.
8	7	++	Bleeding commenced 4th day post-exposure	0/7	2.7	3.0	*E. coli* *S. fecalis* *C. perfringens* *Proteus* sp. *Lactobacillus* sp.
	6	*E. tenella* plus *E. coli*, *C. perfringens* and *S. fecalis*	Bleeding commenced 4th day post-exposure	1/6	3.0	3.0	*E. coli* *S. fecalis* *C. perfringens* *Proteus* sp. *Lactobacillus* sp.
	6	*E. tenella* plus *E. coli*	Mild bleeding commenced on 4th day post-exposure	0/6	2.5	3.0	*E. coli* *S. fecalis* *C. perfringens* *Lactobacillus* sp. *Proteus* sp.

Table IX (Continued)

Trial Number	Number of Chickens	Exposure Pattern with *E. tenella*	Clinical Signs	Mortality	Macroscopic Grading of Lesions (Mean)	Microscopic Grading of Parasitism (Mean)	Dominant Species of Microflora Isolated
8	7	Only *E. coli*, *C. perfringens*, *S. fecalis*	Nil	0/7	0.0	0.0	*E. coli* *S. fecalis* *Lactobacillus* sp. *C. perfringens* *Bacteroides* sp. *Proteus* sp.
	7	*E. coli* alone	Nil	0/7	0.0	0.0	*E. coli* *S. fecalis* *Lactobacillus* sp. *Bacteroides* sp. *C. perfringens* *C. albicans*
	6	--	Nil	0/6	0.0	0.0	*E. coli* *S. fecalis* *Lactobacillus* sp. *Bacteroides* sp. *C. perfringens*

[a] ++ indicates oral inoculation with 100,000 surface sterilized oocysts of *E. tenella*.

[b] -- indicates no oocysts of *E. tenella* were administered.

Table X

Occurrence of *Clostridium perfringens* in Three Weeks Old Specific Pathogen-Free and Conventional Chickens Exposed to *Eimeria tenella*

Environment	Gross Lesion Score	Number of Colonies Developing per a Standard Loop of Inoculum from the Caecum	
		Exposed	Non-exposed Controls
Conventional	3.0	30	0
SPF	3.0	100	55
Conventional	4.0	30	0
Conventional	1.0	0	0
SPF	3.0	100	25
Conventional	4.0	6	1
SPF	3.0	300	50
Conventional	3.0[a]	150	
	2.5[b]	120	
	2.7[c]	51	35

Table X (Continued)

Environment	Gross Lesion Score	Number of Colonies Developing per a Standard Loop of Inoculum from the Cecum	
		Exposed	Nonexposed Controls
Conventional	0.0[d]	50	
	0.0[e]	15	
SPF	2.0[a]	160	
	2.5[b]	74	
	1.66[c]	45	19
	0.00[d]	0	
	0.00[e]	14	

[a]Chickens exposed with E. tenella oocysts plus C. perfringens, E. coli and S. fecalis.

[b]Chickens exposed with E. tenella and E. coli.

[c]Chickens exposed with E. tenella alone.

[d]Chickens exposed with E. coli, C. perfringens and S. fecalis.

[e]Chickens exposed with E. coli alone.

Table XI

Mean Packed Cell Volume (PCV) of Bacteria-, Fungi-, and PPLO-Free, Specific Pathogen-Free (SPF) and Conventional Chickens Exposed to *Eimeria tenella*

Environment	Mean Gross Lesion Score	Mean PCV Before Exposure	Mean PCV at 7th Day Post-Infection
Bacteria-, fungi-, PPLO-free	0 0 0	28 24 28.5	26 29 29
Monocontaminated with *C. albicans*	±[a]	27	28
Monocontaminated with *E. coli*	1.0	28	27
Monocontaminated with *S. fecalis*	1.0	27	24
Monocontaminated with *Lactobacillus* sp.	1.0	19	18.5
Polycontaminated with *Lactobacillus* sp. and *Mucor* sp.	2.0	37 30	28 17.5
Monocontaminated with anaerobic fecal streptococci	0	17	21.5
Monocontaminated with anaerobic fecal streptococci plus killed *E. coli*, *S. fecalis* and *C. perfringens*	0	27	31

Table 1 (Continued)

Environment	Mean Gross Lesion Score	Mean PCV Before Exposure	Mean PCV at 7th Day Post-Infection
Monocontaminated with *Bacteroides* sp.	2.0	35	25.5
Monocontaminated with *E. coli*	0 (Control)	28	31
Polycontaminated with *S. fecalis* and *E. coli*	1.0	27	25
Polycontaminated with *S. perfringens* and *E. coli*	2.0	21	16
Polycontaminated with *E. coli*, *C. perfringens* and *S. fecalis*	0 (Control)	26	27
Polycontaminated with *S. fecalis* and *E. coli*	0 (Control)	27	25
Polycontaminated with *Lactobacillus* sp. and *Mucor* sp.	0 (Control)	30	28
SPF	2.0	32	33
	0 (Control)	32	33

Table XI (Continued)

Environment	Mean Gross Lesion Score	Mean PCV Before Exposure	Mean PCV at 7th Day Post-Infection
Conventional	2.0	30	31
	0 (Control)	30	31
SPF	2.25	29	27
	0 (Control)	29	30
Conventional	2.18	32	20
	0 (Control)	32	30
SPF	2.0	31	20
	0 (Control)	31	28
Conventional	3.0	36	20
	0 (Control)	36	28

Table XI (Continued)

Environment	Mean Gross Lesion Score	Mean PCV Before Exposure	Mean PCV at 7th Day Post-Infection
Polycontaminated with *Clostridium tetanoides* and *Aspergillus* sp.	2.0	27	24
Polycontaminated with *Lactobacillus* sp. and *S. fecalis*	1.5	19	26.5
SPF	2.75	23	29
	0 (Control)	23	32
Conventional	3.0	25	20
	0 (Control)	25	31
SPF	2.75	29	16.5
	0 (Control)	29	31
Conventional	2.6	30	11
	0 (Control)	30	29

Table XI (Continued)

Environment	Mean Gross Lesion Score	Mean PCV Before Exposure	Mean PCV at 7th Day Post-Infection
SPF	1.66[b]	26	22.0
	2.00[c]	26	18.5
	2.50[d]	26	20.0
	0.00[e]	26	29.0
	0.00[f]	26	32.0
	0.00[g]	26	31.0
Conventional	2.7[b]	29.5	20.0
	3.0[c]	29.5	19.0
	2.5[d]	29.5	28.0
	0[e]	29.5	30.0

Table XI (Continued)

Environment	Mean Gross Lesion Score	Mean PCV Before Exposure	Mean PCV at 7th Day Post-Infection
Conventional	0[f]	29.5	30.0
	0[g]	29.5	28.5

[a]± denotes doubtful cases.

[b]Chickens exposed with E. tenella alone.

[c]Chickens exposed with E. tenella, E. coli, S. fecalis and C. perfringens.

[d]Chickens exposed with E. tenella and E. coli.

[e]Chickens exposed with E. coli only.

[f]Chickens exposed with E. coli, S. fecalis and C. perfringens.

[g]Unexposed controls.

Table XII

Total Serum Proteins and Serum Protein Fractions in Infected and Noninfected Conventional, Specific Pathogen-Free (SPF) and Bacteria-, Fungi- and PPLO-Free Chickens

Group	Infected/ Noninfected	Total Serum Proteins[a]	Albumin[b]	Alpha-1 Globulin[b]	Alpha-2 Plus Beta Globulin[b]	Gamma Globulin[b]
Conventional	Noninfected	3.03	2.26	0.05	0.27	0.46
	Infected	2.99	1.68	0.42	0.19	0.70
Conventional	Noninfected	7.45	5.13	0.47	0.77	1.08
	Infected	5.73	3.14	0.55	0.68	1.36
Conventional	Noninfected	3.00	1.875	0.375	0.375	0.375
	Infected	0.198	0.108	0.030	0.020	0.040
SPF	Noninfected	2.04	1.47	0.15	0.03	0.39
	Infected	1.15	0.70	0.10	0.02	0.33
SPF	Noninfected	7.07	5.07	0.72	0.70	1.27
	Infected	4.33	3.04	0.18	0.28	0.83
SPF	Noninfected	2.92	1.90	0.20	0.41	0.41
	Infected	1.52	0.96	0.13	0.17	0.26
Bacteria-fungi- and PPLO-free	Noninfected	5.03	3.52	0.35	0.58	0.58
	Infected	7.10	5.56	0.44	0.66	0.44

Table XII (Continued)

Group	Infected/ Noninfected	Total Serum Proteins[a]	Albumin[b]	Alpha-1 Globulin[b]	Alpha-2 Plus Beta Globulin[b]	Gamma Globulin[b]
Bacteria-, fungi- and PPLO-free	Noninfected	5.04	3.50	0.42	0.70	0.42
	Infected	6.01	3.16	0.92	0.84	1.09

[a]Grams per 100 ml of serum.

[b]Grams per 100 ml of serum, calculated from total serum proteins.

APPENDIX B

Figure 1. Ceca of 3 week-old specific pathogen-free chicken exposed to Eimeria tenella, 7th day post-inoculation. Macroscopic grading of the lesion ++++.

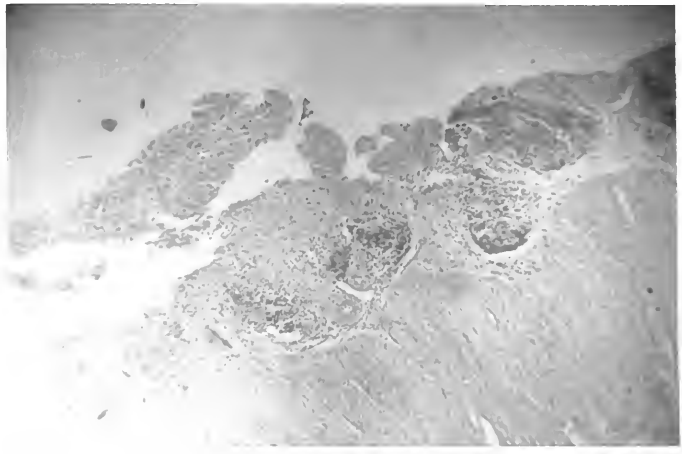

Figure 2. Photomicrograph of a transverse section of cecum of 3 week-old specific pathogen-free chicken exposed to Eimeria tenella showing denudation of mucosa, and large 2nd generation schizonts. Hematoxylin-eosin stain. X125. Microscopic grading of parasitism ++++.

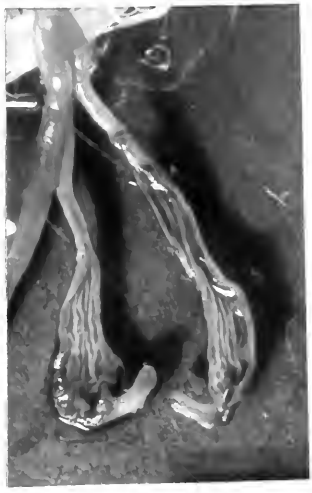

Figure 3. Ceca of 3 week-old bacteria-, fungi- and PPLO-free chicken exposed to Eimeria tenella, 7th day post-inoculation. Macroscopic grading of the lesion 0.

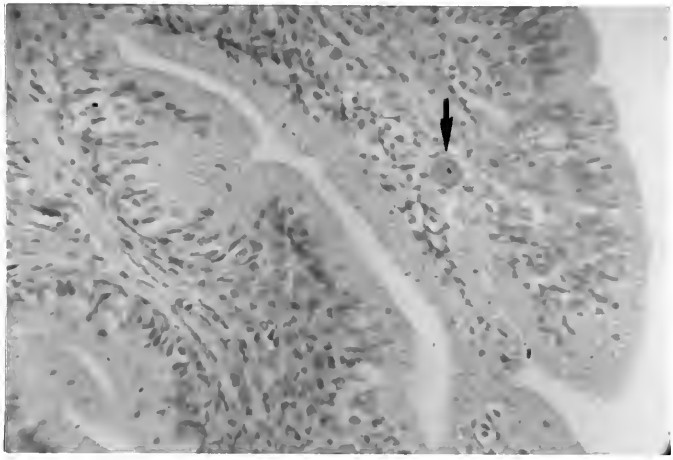

Figure 4. Photomicrograph of a transverse section of cecum of 3 week-old bacteria-, fungi and PPLO-free chicken exposed to Eimeria tenella showing a single coccidium and no denudation of mucosa or other tissue damage. Hematoxylin-eosin stain. X400.

Figure 5. Ceca of 3 week-old bacteria-, fungi- and PPLO-free chicken exposed to Eimeria tenella and Clostridium perfringens. Macroscopic grading of lesion ++++.

BIBLIOGRAPHY

Anon. 1965. ARS-USDA. Losses in agriculture. Agric. Handbook No. 291. U.S. Gov't Printing Office, Washington, D.C., p. 80.

Balish, E. and A. W. Phillips. 1966. Growth, morphogenesis and virulence of Candida albicans after oral inoculation in the germ-free and conventional chick. J. Bact. 91:1736-1742.

Ball, S. J. 1959. Antibiotics in chemotherapy of caecal coccidiosis in chickens. J. Comp. Path. and Therap. 69:327-333.

Balzam, N. 1937a. Aseptische zucht von tiern. I. Appareillage et methode. II. Aseptische zucht von haken worm mit vitamin haltigen und vitamin f. futter. Acta. Biol. Exptal. (Warsau) 11:43-50. Ber. Biol. 43:545.

Balzam, N. 1937b. Elevage aseptique des animaux. I. Appareillage et methode. II. Elevage aseptique des poules maintenues aux regimes complets et deficients. Ann. Physiol. Physicochim. Biol. 13:370-385.

Bankowski, R. A., D. E. Stover, and S. L. Jamison. 1959. Coccidiosis immunization at a poultry testing project. Ann. Proc. U.S. Livestock Sanitary As. Dec. p. 5.

Barnes, D. M. and H. W. Moon. 1964. Enterotoxemia in pigs due to Clostridium perfringens type C. J. Am. Vet. Med. As. 144:1391-1394.

Barnes, E. M. and C. S. Impey. 1968. Anaerobic gram negative, non-sporing bacteria from the ceca of poultry. J. Appl. Bact. 31:530-541.

Becker, E. R. 1934. Coccidia and Coccidiosis of Domesticated Game, and Laboratory Animals and of Man. Iowa State College Press, Ames, Iowa.

Becker, E. R. 1956. Apropos oocyst measurements. J. Parasit. 42 (Suppl.):24.

Bedrnik, P. 1967. Development of sexual stages and oocysts from second generation Eimeria tenella merozoites in tissue cultures. Folia Parasit. 14:364.

Bedrnik, P. 1969. Some results and problems of cultivation of Eimeria tenella in tissue cultures. Acta. Vet. 38:31-35.

Bergeland, H. S. 1965. Studies of a porcine enterotoxemia caused by Clostridium perfringens type C. Doctoral dissertation, University of Minnesota.

Bertke, E. M. 1963. Renal function during the course of Eimeria tenella infection. J. Parasit. 49:937-942.

Biester, H. E. and L. H. Schwarte (eds.). 1965. Diseases of Poultry. Iowa State University Press, Ames, Iowa, p. 823-858.

Boggs, D. R., P. A. Chervenick, J. C. Marsh, H. I. Pilgrim, G. E. Cartwright, and M. M. Wintrobe. 1967. Granulocytopoiesis in germ-free mice. Proc. Soc Exp. Biol. Med. 125:325-330.

Boney, W. A. 1940. The efficacy of methyl bromide as a fumigant for the prevention of cecal coccidiosis in chickens. Am. J. Vet. Res. 9:210-214.

Brackett, S. and A. Bliznick. 1949. The effect of small doses of drugs on oocyst production of infections with Eimeria tenella. Ann. N. Y. Acad. Sci. 52: 595-610.

Brackett, S. and A. Bliznick. 1952. The reproductive potential of five species of coccidia of the chicken as demonstrated by oocyst production. J. Parasit. 38:133-139.

Bradley, R. E., H. Botero, J. Johnson, and W. M. Reid. 1967. Gnotobiotic poultry in plastic film isolators and some applications to parasitological research. Exp. Parasit. 21:403-413.

Bradley, R. E., J. Johnson, and W. M. Reid. 1964. Apparent obligate relationship between Histomonas meleagridis and Escherichia coli in producing disease. J. Parasit. 50:51.

Bradley, R. E. and W. M. Reid. 1966. Histomonas meleagridis and several bacteria as agents of infectious enterohepatitis in gnotobiotic turkeys. Exp. Parasit. 19:91-101.

Breed, R. S., E. G. D. Murray, and N. R. Smith. 1957. Bergey's Manual of Determinative Bacteriology. 7th ed. Williams and Wilkins Co., Baltimore, Maryland, p. 1094.

Briggs, B. D. (ed.). 1968. Coccidiosis of Chickens. Norwich Animal Industry, Inc., Norwich, New York, p. 1-32.

Britton, W. M., C. H. Hill, and C. W. Barber. 1964. A mechanism of interaction between dietary protein levels and coccidiosis in chicks. J. Nutrit. 82: 306-310.

Burns, W. C. 1959. The lethal effects of Eimeria tenella extracts on rabbits. J. Parasit. 45:38-46.

Challey, J. R. 1962. The role of bursa of Fabricius in adrenal response and mortality due to Eimeria tenella infection in the chicken. J. Parasit. 43:352-357.

Challey, J. R. and W. C. Burns. 1959. The invasion of the cecal mucosa by Eimeria tenella sporozoites and their transport by macrophages. J. Prot. 6:238-241.

Cheissin, E. M. 1958. Cytologische untersuchungen verschiedener stadien des lebens-zyklus der kaninchen coccidien. I. Eimeria intestinalis. Arch. Protistenk. 102:265-290.

Clark, D. T., C. K. Smith, and R. B. Dardas. 1962. Pathological and immunological changes in gnotobiotic chickens due to Eimeria tenella. Poultry Sci. 41:1635-1636.

Coates, M. E., J. E. Ford, and G. F. Harrison. 1968. Intestinal synthesis of some vitamins of the B complex in chickens. In I. Hiyakawa and T. D. Luckey (eds.), Advances in Germ-Free Research and Gnotobiology. The Chemical Rubber Company Press, Cleveland, Ohio, p. 110-111.

Cohendy, M. 1912. Expériences sur la vie sans microbes. Ann. Inst. Pasteur. 26:106-137.

Cohendy, M. and E. Wollman. 1914. Présentée par M. Roux. Bacteriologie. Expériences sur la vie sans microbes. Elevage aseptique de cobayes. Compt. Rend. Acad. Sci. 158:1283-1284.

Cook, F. D., R. M. Blakely, H. I. McGregor, and R. W. Anderson. 1954. The effect of antibiotics on the intestinal microflora of turkey poults. Poultry Sci. 33:38-40.

Cuckler, A. C. 1957. The effect of nicarbazin on the development of immunity to reinfection with coccidia in poultry. Merck Poultry Nutrition and Health Symposium, St. Louis, Missouri, p. 22.

Cuckler, A. C., L. R. Chapin, C. M. Malanga, E. F. Rogers, H. J. Becker, R. L. Clark, W. J. Leanza, A. A. Pessolano, T. Y. Shen, and L. H. Sarett. 1958. Antiparasitic drugs. II. Anticoccidial activity of 4,5-imidazoldicar boxamide and related compounds. Proc. Soc. Exp. Biol. and Med. 98:167-170.

Daugherty, J. W. and C. A. Herrick. 1952. Cecal coccidiosis and carbohydrate metabolism in chickens. J. Parasit. 38:299-304.

Davies, S. F. M. and L. P. Joyner. 1962. Infection of the fowl by the parenteral inoculation of ooc. of Eimeria. Nature 194:996-997.

Davies, S. F. M., L. P. Joyner, and S. B. Kendall. 1963. Coccidiosis. Oliver and Boyd, Edinburgh.

Davies, S. F. M. and S. B. Kendall. 1955. An experimental assessment of the value of nitrofurazone used continuously as a coccidiostatic drug. Vet. Rec. 67: 867-870.

Doflein, F. and E. Reichenow. 1953. Lehrbuch der protozoenkunde, Jena: Fischer Verlag. p. 1213.

Doll, J. P. and C. K. Franker. 1963. Experimental histomoniasis in gnotobiotic turkeys. I. Infection and histopathology of the bacteria-free host. J. Parasit. 49:411-414.

Doll, J. P., P. C. Trexler, L. I. Reynolds, and G. R. Bernard. 1963. The use of peracetic acid to obtain germ-free invertebrate eggs for gnotobiotic studies. Am. Midl. Nat. 69:231-239.

Domermuth, C. H. and W. B. Gross. 1969. A medium for isolation and tentative identification of fecal streptococci, and their role as avian pathogens. Avian Dis. 13:394-399.

Doran, D. J. 1970. Eimeria tenella: from sporozoites to oocysts in cell culture. Proc. Helm. Soc. Wash. 37:84-91.

Doran, D. J. and M. M. Farr. 1962. Excystation of the poultry coccidium, Eimeria acervulina. J. Prot. 9:154-161.

Doran, D. J. and M. M. Farr. 1965. Susceptibility of 1- and 3-day-old chicks to infection with the coccidium, Eimeria acervulina. J. Prot. 12:160-166.

Dubos, R. J. and R. W. Schaedler. 1962. The effect of diet on the fecal bacterial flora of mice and on their resistance to infection. J. Exp. Med. 115: 1161-1172.

Dubos, R. J., R. W. Schaedler, R. Costello, and P. Hoet. 1965. Indigenous and autochthonous flora of the gastrointestinal tract. J. Exp. Med. 122:67-75.

Dunkley, M. J. W. 1968. Laboratory trials with buquinolate--a new broad-spectrum coccidiostat for poultry. Vet. Rec. 83:30-34.

Edgar, S. A. 1941. The development of the protozoan parasite Eimeria tenella Railliet and Lucet 1891 in domestic fowl. Doctoral dissertation, University of Wisconsin Memorial Library, Madison.

Edgar, S. A. 1955. Sporulation of oocysts at specific temperatures and notes on the prepatent period of several species of avian coccidia. J. Parasit. 41:214-216.

Edgar, S. A. and C. A. Herrick. 1944. Feeding habits in relation to the severity of cecal coccidiosis, Eimeria tenella. Poultry Sci. 23:30-35.

Edgar, S. A. and C. T. Seibold. 1964. A new coccidium of chickens Eimeria mivati sp. n. (Protozoa: Eimeriidae) with details of its life-history. J. Parasit. 50: 193-204.

Eyssen, H. and P. DeSomer. 1967. Effects of Streptococcus fecalis and a filterable agent on the growth and nutrient absorption in gnotobiotic chicks. Poultry Sci. 46:323-333.

Farr, M. M. and E. E. Wehr. 1945. Sulfamerazine therapy in experimental cecal coccidiosis of chickens. J. Parasit. 31:353-358.

Field, H. I. and E. A. Gibson. 1955. Studies on piglet mortality. II. Clostridium welchii infection. Vet. Rec. 67:31-35.

Fish, F. F. 1931. Quantitative and statistical analysis of infections with Eimeria tenella in the chicken. Am. J. Hyg. 14:560-576.

Forbes, M. and J. T. Park. 1959. Growth of germ free and conventional chicks: effect of diet, dietary penicillin, and bacterial environment. J. Nutrit. 67: 69-84.

Forbes, M., J. T. Park, and M. Lev. 1959. Role of the intestinal flora in the growth response of chicks to dietary penicillin. Ann. N. Y. Acad. Sci. 78: 321-327.

Fowler, E. H. and N. W. Rohovsky. 1970. Enzyme histochemistry of the small intestine in germ free and specific pathogen free cats inoculated with feline infectious enteritis (panleukopenia) virus. Am. J. Vet. Res. 31:2055-2060.

Franker, C. K. and J. P. Doll. 1964. Experimental histomoniasis in turkeys. II. Effects of some cecal bacteria on pathogenesis. J. Parasit. 50:636-640.

Freeman, B. M. 1970. Carbohydrate stores in chickens infected with Eimeria tenella. Parasitology 61: 245-251.

Gardiner, J. L. 1955. The severity of cecal coccidiosis infection in chickens as related to the age of the host and the number of oocysts ingested. Poultry Sci. 34:415-420.

Gardiner, J. L. and M. M. Farr. 1954. Nitrofurazone for the prevention of experimentally induced Eimeria tenella infections in chickens. J. Parasit. 40: 42-49.

Gardiner, J. L. and D. K. McLoughlin. 1963. Drug resistance in Eimeria tenella. III. Stability of resistance to glycarbylamide. J. Parasit. 49:657-659.

Glimstedt, G. 1936. Der stoffwechsel bakterienfrier tiere. I. Allgemeine methodik. Skand. Arch. Physiol. 73: 48-62.

Goodrich, H. P. 1944. Coccidian oocysts. Parasitology 36:72-79.

Gordon, H. A. 1960. The germ free animal. Its use in the study of "physiologic" effects of the normal microbial flora on the animal host. Am. J. Digest Dis. 5:841-867.

Gordon, H. A., M. Wagner, T. D. Luckey, and J. A. Reyniers. 1959. An encephalo-meningeal syndrome selectively affecting newly hatched germfree and monocontaminated chickens. J. Inf. Dis. 105:31-37.

Graber, C. D., R. W. Moore, M. Suzuki, W. E. Redmond, R. M. O'Neil, and B. M. Lockhart. 1966. Autochthonous intestinal bacterial flora and cholesterol levels in specific pathogen-free swine fed high lipid and high sucrose diets. J. Bact. 92:1290-1297.

Griesemer, R. A., J. P. Gibson, and D. S. Elasser. 1963. Congenital ascariasis in gnotobiotic dogs. J. Am. Vet. Med. As. 143:962-964.

Gustafsson, B. E. 1948. Germ free rearing of rats. Acta. Path. Microbiol. Scand. (Suppl.) 73:1-130.

Hegde, K. S., W. M. Reid, J. Johnson, and H. E. Womack. 1969. Pathogenicity of Eimeria brunetti in bacteria free and conventional chickens. J. Parasit. 55: 402-405.

Hentges, D. J. 1967. Influence of pH on the inhibitory activity of formic and acetic acids for Shigella. J. Bact. 93:2029-2030.

Hentges, D. J. 1969. Inhibition of Shigella flexneri by the normal intestinal flora. II. Mechanism of inhibition by coliform organisms. J. Bact. 97: 513-517.

Herrick, C. A. 1936. Organ specificity of the parasite Eimeria tenella. J. Parasit. 22:226-227.

Herrick, C. A., C. E. Holmes, and D. L. Deciusti. 1942. The experimental use of organic sulfur compounds for the prevention of cecal coccidiosis in chickens. Am. J. Vet. Res. 3:117-127.

Herrick, C. A., G. L. Ott, and C. E. Holmes. 1936. Age as a factor in the development of resistance of the chicken to the effect of the protozoan parasite Eimeria tenella. J. Parasit. 22:262-272.

Hibbert, L. E., D. M. Hammond, and J. R. Simmons. 1969. The effects of pH, buffers, bile, and bile acids on excystation of sporozoites of various Eimeria species. J. Prot. 16:441-444.

Holmes, C. E., C. A. Herrick, and G. L. Ott. 1937. Studies in coccidiosis: calcium carbonate additions and coccidia. Poultry Sci. 16:335-339.

Horton-Smith, C. 1947. Coccidiosis. Some factors influencing its epidemiology. Vet. Rec. 59:645-646.

Horton-Smith, C., J. Beattie, and P. L. Long. 1961. Resistance to Eimeria tenella and its transference from one cecum to the other in individual fowls. Immunology 4:111-121.

Horton-Smith, C. and P. L. Long. 1963. Coccidia and coccidiosis in domestic fowl and turkey. Adv. Parasit. 1:68-104.

Horton-Smith, C., P. L. Long, and A. E. Pierce. 1963. Behavior of invasive stages of Eimeria tenella in the immune fowl (Gallus domesticus). Exp. Parasit. 14:66-74.

Huhtanen, C. N. and J. M. Pensack. 1965. The development of the intestinal flora of the young chick. Poultry Sci. 44:825-830.

Ikeda, M. 1955a. Factors necessary for Eimeria tenella infection of the chicken. II. Influence of the pancreatic juice on infection. Jap. J. Vet. Sci. 17:225-230.

Ikeda, M. 1955b. Factors necessary for Eimeria tenella infection of chickens. Jap. J. Vet. Sci. 18:25-30.

Ikeda, M. 1956. Factors necessary for Eimeria tenella infection of the chicken. IV. Investigations of the site of action of the pancreatic juice on infection. Jap. J. Vet. Sci. 18:45-53.

Ikeda, M. 1960. Factors necessary for E. tenella infection of the chicken. VI. Excystation of oocyst in vitro. Jap. J. Vet. Sci. 22:27-41.

Jankiewicz, H. A. and R. H. Scofield. 1934. The administration of heated oocysts of Eimeria tenella as a means of establishing resistance and immunity to cecal coccidiosis. J. Am. Vet. Med. As. 84:507-526.

Jeffers, T. K. and G. E. Wagenbach. 1969. Sex differences in embryonic response to Eimeria tenella infection. J. Parasit. 55:949-951.

Johansson, K. R. and W. B. Sarles. 1948. Bacterial population changes in the ceca of young chickens infected with Eimeria tenella. J. Bact. 56:635-647.

Johansson, K. R., W. B. Sarles, and S. K. Shapiro. 1948. The intestinal microflora of hens as influenced by various carbohydrates in a biotin-deficient diet. J. Bact. 56:619-634.

Johnson, G. R., A. Koestner, and M. W. Rohovsky. 1967. Experimental feline infectious enteritis in the germ free cat. An electron microscopic study. Path. Vet. 4:275-288.

Johnson, J. 1971. The development of Ascaridia galli in germ-free chickens. J. Parasit. 56:174.

Johnson, J. and W. M. Reid. 1970. Anticoccidial drugs: lesion scoring techniques in battery and floor-pen experiments with chickens. Exp. Parasit. 28:30-36.

Johnson, W. T. 1927. Immunity or resistance of the chickens to coccidial infection. Oregon Agric. Exp. Sta. Bull. 230.

Jones, D. and E. Griffiths. 1964. The use of thin soil sections for the study of soil microorganisms. Plant Soil 20:232-240.

Jones, E. E. 1932. Size as a species characteristic in coccidia. Variations under diverse conditions of infection. Arch. Protistenk. 76:130.

Joyner, L. P. 1964. Coccidiosis in the domestic fowl. A review of the disease in Britain and its chemotherapeutic control during the past decade. Vet. Bull. 34:311-315.

Joyner, L. P. 1970. Coccidiosis: problems arising from the development of anticoccidial drug resistance. Expt. Parasit. 28:122-128.

Karmann, P. and I. Presch. 1933. Wird die kokzidiose der küken durch eine oder durch mehrere Eimeria arten herrvorgerufen. Tierarzt Rdsdr. 39:529-531.

Kemp, R. L., W. M. Reid, and J. Johnson. 1971. Delayed development of endogenous stages of Eimeria tenella in germ-free chicks. The American Society of Parasitologists. August 23-27, University of California, Los Angeles, California. Programs and Abstracts, p. 42.

Kohler, E. M. and R. F. Cross. 1969. Studies of Escherichia coli in gnotobiotic pigs. VI. Effects of feeding bacteria-free filtrates of broth cultures. Canad. J. Comp. Med. 33:173-177.

Kohler, E. M. and R. F. Cross, 1971. Feeding bacteria-free whole cell lysates of Escherichia coli to gnotobiotic pigs and the effects of giving antiserums. Am. J. Vet. Res. 32:739-748.

Kolacz, J. W., R. B. Wescott, and A. R. Dommert. 1970. Microflora of Hormel miniature swine. Am. J. Vet. Res. 31:1173-1178.

Küster, E. 1912. Die keim freie züchtung von saügetieren und ihre bedeutung für die er for schung der korperfunktionen. Zentr. Baketeriol. Parasitenk. 54: 55-58.

Layton, H. W. and K. L. Simkins. 1971. Growth and survival of germ free and conventional chicks inoculated with Mycoplasma gallisepticum. Am. J. Vet. Res. 32:1269-1274.

Lev, M. and C. A. E. Briggs. 1956. Gut flora of the chick. II. The establishment of flora. J. Appl. Bact. 19:224-230.

Lev, M., C. A. E. Briggs, and M. E. Coates. 1957. The gut flora of the chick. III. Differences in cecal flora between "infected," "uninfected," and penicillin fed chicks. Brit. J. Nutrit. 11:364-372.

Lev, M. and M. Forbes. 1959. Growth response to dietary penicillin of germ-free chicks and of chicks with defined intestinal flora. Brit. J. Nutrit. 13: 78-84.

Levine, L. and C. A. Herrick. 1954. The effect of the protozoan Eimeria tenella on the ability of the chicken to do muscular work when its muscles are stimulated directly or indirectly. J. Parasit. 40:525-531.

Levine, L. and C. A. Herrick. 1957. Effects of cecal coccidia on the ability of Plymouth Rocks to do muscular work. Poultry Sci. 36:24-29.

Levine, N. D. 1961. Protozoan Parasites of Domestic Animals and Man. Burgess Publishing Company, Minneapolis, Minnesota, p. 158-253.

Levine, N. D. 1963. Coccidiosis. Ann. Rev. Microbiol. 17:179-198.

Levine, N. D. 1969. Uniform terminology for sporozoan protozoa. Prog. Prot. 3rd Int. Cong. Prot., Leningrad, p. 340.

Levine, N. D. 1970. Taxonomy of the sporozoa. J. Parasit. 56:208-209.

Levine, P. P. 1940. Subclinical coccidial infection in chickens. Cornell Vet. 30:127-132.

Levine, P. P. 1942. Excystation of coccidial oocysts of the chicken. J. Parasit. 28:426-428.

Levine, P. P. and C. W. Barber. 1947. The comparative efficacy of some coccidiostatic agents against experimental infection with Eimeria tenella. Cornell Vet. 37:155-159.

Long, P. L. 1965. Development of Eimeria tenella in avian embryos. Nature 208:509-510.

Long, P. L. 1966. The growth of some species of Eimeria in avian embryos. Parasitology 56:575-581.

Long, P. L. 1971. Schizogony and gametogony of Eimeria tenella in the liver of chick embryos. J. Prot. 18:17-20.

Lotze, J. C. and R. G. Leek. 1960. Some factors involved in excystation of the sporozoites of three species of sheep coccidia. J. Parasit. 46 (Suppl.):46.

Lotze, J. C. and R. G. Leek. 1969. Observations on Eimeria tenella in feces of inoculated chickens. J. Prot. 16:496-498.

Luckey, T. D. 1963. Germfree Life and Gnotobiology. Academic Press, New York.

Lynch, J. E. 1961. A new method for the primary evaluation of anticoccidial activity. Am. J. Vet. Res. 22:324-326.

Mann, T. B. 1947. Chick rearing. X. Roughage and protein as dietary factors influencing coccidiosis in chicks, with notes on the limitation of sulphamethazine in the control of coccidiosis. J. Agri. Sc. 37:145-151.

Matsuoka, T., M. E. Callender, and R. F. Shumard. 1969. Embryonic bovine trachea cell line for in vitro cultivation of Eimeria tenella. Am. J. Vet. Res. 30:1119-1122.

Mayhew, R. L. 1934. Studies on coccidiosis. VI. Effect of early attack on egg production. Poultry Sci. 13:148-154.

Mayhew, R. L. 1937. Studies on coccidiosis. IX. Histopathology of the cecal type in the chicken. Trans. Am. Micros. Soc. 56:431-446.

McLaren, D. J. 1969. Observations on the fine structural changes associated with schizogony and gametogony in Eimeria tenella. Parasitology 59:563-574.

McLaren, D. J. and G. E. Paget. 1968. A fine structural study on the merozoite of Eimeria tenella with special reference to the conoid apparatus. Parasitology 58:561-571.

McLaughlin, M. M., M. P. Dacquisto, D. P. Jacobus, M. Forbes, and P. E. Parks. 1958. The effect of the germ-free state on survival of the ten day old chick after X-radiation. Radiation Res. 9:147.

McLoughlin, D. K. 1970. Coccidiosis: experimental analysis of drug resistance. Expt. Parasit. 28: 129-136.

McLoughlin, D. K. and J. L. Gardiner. 1961a. Drug-resistance in Eimeria tenella. I. The experimental development of a glycarbylamide-resistant strain. J. Parasit. 47:1001-1006.

McLoughlin, D. K. and J. L. Gardiner. 1961b. Zoalene tolerance by Eimeria tenella. J. Parasit. 47 (Suppl.):46.

McLoughlin, D. K. and J. L. Gardiner. 1962. Drug-resistance in Eimeria tenella. II. The experimental development of a zoalene-resistant strain. J. Parasit. 53:930-932.

Meyer, R. C., E. H. Bohl, and E. M. Kohler. 1964. Procurement and maintenance of germ-free swine for microbiological investigations. Appl. Microbiol. 12:295-300.

Meyer, R. C., H. E. Rhoades, and S. P. Saxena. 1967. The effect of 5 E. coli serotypes on gnotobiotic swine. Proc. U.S. Livestock San. As., p. 345-348.

Meyer, R. C., S. P. Saxena, and H. E. Rhoades. 1971. Polyserositis induced by Escherichia coli in gnotobiotic swine. Infection and Immunity 3:41-44.

Meynell, G. G. 1963. Antibacterial mechanisms of the mouse gut. II. The role of Eh and volatile fatty acids in the normal gut. Brit. J. Exp. Path. 44: 209-219.

Miyakawa, M. 1954. Long term germ free rearing of guinea pigs. Trans. Soc. Path. Japon 43:450-452.

Monné, L. and G. Hönig. 1954. On the properties of the shells of the coccidian oocysts. Ark. Zool. Stockholm 7:251-256.

Musajev, M. A. and A. M. Surkova. 1970. Some indices of the nitrogen metabolism in the liver of chickens under experimental coccidiosis. J. Parasit. 56: 451-452.

Naqi, S. A., C. F. Hall, and D. H. Lewis. 1970a. Microflora of turkeys: comparison of apparently healthy and bluecomb-infected turkey poults. Avian Dis. 15:14-21.

Naqi, S. A., D. H. Lewis, and C. F. Hall. 1970b. The intestinal microflora of turkeys. Avian Dis. 14: 620-625.

Natt, M. P. 1959. The effect of cecal coccidiosis on the blood cells of the domestic fowl. III. The changes in the leukocyte picture during the course of the infection. Exp. Parasit. 8:182-187.

Natt, M. P. and C. A. Herrick. 1955. The effects of cecal coccidiosis on the blood cells of the domestic fowl. I. A comparison of the changes in the erythrocyte

count resulting from hemorrhage in infection and mechanically bled birds. Poultry Sci. 34:1100-1106.

Newton, W. L. 1965. Methods in germ-free animal research. In W. I. Gay (ed.), Methods of Animal Experimentation, Vol. I. Academic Press, New York, p. 215-271.

Newton, W. L., L. V. Reardon, and A. M. DeLeva. 1960. A comparative study of the subcutaneous inoculation of germ-free and conventional guinea pigs with two strains of Trichomonas vaginalis. Am. J. Trop. Med. Hyg. 9:56-61.

Newton, W. L., P. P. Weingstein, and M. F. Jones. 1959. A comparison of the development of some rat and mouse helminths in germ-free and conventional guinea pigs. Ann. N. Y. Acad. Sci. 78:290-397.

Norcross, M. A. and F. V. Washko. 1970. Coccidiosis: laboratory confirmation of clinical disease. Exp. Parasit. 28:137-146.

Nyberg, P. A. and D. M. Hammond. 1964. Excystation of Eimeria bovis and other species of bovine coccidia. J. Prot. 11:474-480.

Nyberg, P. A. and S. E. Knapp. 1970a. Scanning electron microscopy of Eimeria tenella oocysts. Proc. Hel Soc. Wash. 37:29-32.

Nyberg, P. A. and S. E. Knapp. 1970b. Effect of sodium hypochlorite on the oocyst wall of Eimeria tenella as shown by electron microscopy. Proc. Helm. Soc. Wash. 37:32-36.

Ogata, M. and Y. Morishita. 1969. Studies on the alimentary flora of pigs. IV. The alimentary flora of pigs infected with hog cholera. Jap. J. Vet. Sci. 31:71-82.

Ott, G. L. 1937. Studies on some relationships between colon bacilli and the protozoan parasite Eimeria tenella of chickens. Doctoral dissertation, University of Wisconsin, Madison, Wisconsin.

Pasteur, L. 1885. Observations relatives á la note précédénte de M. Ducláux. Compt. Rend. Acad. Sci. 100:68.

Patnaik, B. 1966. Experiments with Eimeria tenella Railliet and Lucet, 1891 in chickens by parenteral routes of infection. Ind. Vet. J. 43:868-880.

Pattillo, W. H. 1959. Invasion of the cecal mucosa of the chickens by sporozoites of Eimeria tenella. J. Parasit. 45:253-258.

Patton, W. H. 1965. Eimeria tenella: cultivation of the asexual stages in cultured animal cells. Science 150:767-769.

Pellérdy, L. 1961. On the occurrence of coccidium strains resistant to chemicals. Magyar Allatorvosok Lapja 16:422-423.

Pellérdy, L. 1962a. Drug resistance in Eimeria tenella. Vet. Bull. 32:2249.

Pellérdy, L. 1962b. Über das vorkommen sulfonamid-resistenter coccidien stamme. Monatshefte Für Veterinärmedizin 17:346-348.

Phillips, B. P. 1962. Personal communication quoted by W. L. Newton, 1965. Methods in germ-free animal research. In W. I. Gay (ed.), Methods of Animal Experimentation, Vol. I. Academic Press, New York.

Phillips, B. P. 1964. Studies on the ameba-bacteria relationship in amebiasis. III. Induced amebic lesions in the germ-free guinea pig. Am. J. Trop. Med. Hyg. 13:391-395.

Phillips, B. P. and F. Gorstein. 1966. Effects of different species of bacteria on the pathology of enteric amebiasis in mono-contaminated guinea pi Am. J. Trop. Med. Hyg. 15:863-868.

Phillips, B. P. and P. A. Wolfe. 1959. The use of germ-free guinea pigs in studies on microbial inter-relationships in amoebiasis. Ann. N. Y. Acad. Sci. 78:308-314.

Phillips, B. P., P. A. Wolfe, and I. L. Bartgis. 1958. Studies on the ameba-bacteria relationship in amebiasis. II. Some concepts on the etiology of the disease. Am. J. Trop. Med. Hyg. 7:392-399.

Phillips, B. P., P. A. Wolfe, C. W. Rees, H. H. Gordon, W. H. Wright, and J. A. Reyniers. 1955. Studies on the amoeba-bacteria relationship in amoebiasis. Comparative results of the intracecal inoculation of germ-free, mono-contaminated and conventional guinea pigs with Entamoeba histolytica. Am. J. Trop. Med. Hyg. 4:675-692.

Pierce, A. E., P. L. Long, and C. Horton-Smith. 1962. Immunity to Eimeria tenella in young fowls. Immunology 5:129-152.

Pratt, I. 1940. The effect of Eimeria tenella (Coccidia) upon the blood sugar of the chicken. Trans. Am. Micr. Soc. 59:31-37.

Pratt, I. 1941. The effect of Eimeria tenella (Coccidia) upon the glycogen stores of the chicken. Am. J. Hyg. 34:54c-61c.

Rall, G. D., A. J. Wood, R. B. Westcott, and A. R. Dommert. 1970. Distribution of bacteria in feces of swine. ppl. Microbiol. 20:789-792.

Reece, W. O., M. S. Hofstad, and H. J. Swenson. 1968. Establishment and maintenance of an SPF beagle dog colony. Lab. Anim. Care 18:509-512.

Reich, F. 1913. Das kaninchencoccid Eimeria steiedae, nebst einen beitragezur kenntnis von Eimeria falciformis. Arch. Prot. 28:1-42.

Reid, W. M. 1960. The relationship between coccidiostats and poultry flock immunity in coccidiosis control programs. Poultry Sci. 39:1431-1437.

Reid, W. M. and H. Botero. 1967. Growth of the cestode Raillietina cesticillus in bacteria-free chickens. Exp. Parasit. 21:149-153.

Reid, W. M., W. T. Springer, and J. K. Johnson. 1969. Requirements of avian parasites for support by microflora in the intestine. 8th Annual Meeting. Association for Gnotobiotics, June 10-13, Oak Ridge, Tennessee.

Reyniers, J. A. (ed.). 1946. Germ-free life studies. Lobund Reports No. 1. University of Notre Dame, Notre Dame, Indiana.

Reyniers, J. A. (ed.). 1949. Germ-free life studies. Lobund Reports No. 2. University of Notre Dame, Notre Dame, Indiana.

Reyniers, J. A. (ed.). 1960. Germ-free life studies. Lobund Reports No. 3. University of Notre Dame, Notre Dame, Indiana.

Reyniers, J. A. and M. R. Sacksteder. 1959. Tumorigenesis and the germ-free chicken. A preliminary report. Ann. N. Y. Acad. Sci. 78:328-353.

Reyniers, J. A., P. C. Trexler, R. F. Ervin, M. Wagner, T. D. Luckey, and H. A. Gordon. 1949. The need for a unified terminology in germ-free life studies. Lobund Reports 2:149-162.

Reyniers, J. A., M. Wagner, T. D. Luckey, and H. A. Gordon. 1960. Survey of germ-free animals. The White Wyandotte Bantam and White Leghorn chicken. Lobund Reports 3:7-171.

Ripsom, C. A. and C. A. Herrick. 1945. Effects of various sulfa compounds on the protozoan parasite, Eimeria tenella. J. Parasit. 31:98-108.

Rohovsky, M. W. and R. A. Griesemer. 1967. Experimental feline infectious enteritis in the germfree cat. Path. Vet. 4:391-410.

Rose, M. E. 1967a. Immunity to Eimeria tenella and Eimeria necatrix infection in the fowl. II. Influence of the site of infection and the stage of the parasite. II. Cross infection. Parasitology 57:567-583.

Rose, M. E. 1967b. The influence of age of host on infection with Eimeria tenella. J. Parasit. 53: 924-929.

Rosenberg, M. M. 1941. A study of the inheritance of resistance to E. tenella in the domestic fowl. Poultry Sci. 20:472.

Rosenberg, M. M. and W. H. McGibbon. 1948. Selection for hereditary resistance and susceptibility to cecal coccidiosis in the domestic fowl. 8th World's Poultry Congress 1:745.

Sabourdy, M. A. 1965. The need for specification in laboratory animals: suggested terms and definitions. Food Cosmet. Toxicol. 3:19.

Savage, D. C. and R. Dubos. 1968. Alterations in the mouse cecum and its flora produced by antibacterial drugs. J. Exp. Med. 128:97-110.

Savage, D. C., R. Dubos, and R. W. Schaedler. 1968. The gastro intestinal epithelium and its autochthonous bacterial flora. J. Exp. Med. 127:67-76.

Savage, D. C., J. S. MacAllister, and P. C. Davies. 1970. Anaerobic bacteria on the mucous membrane epithelium of murine large bowel. Bact. Proc. 98.

Schaedler, R. W., R. Dubos, and R. Costello. 1965. The development of the bacteria flora in the gastrointestinal tract of mice. J. Exp. Med. 122:59-66.

Scholtyseck, E. 1962. Electron microscope studies on Eimeria perforans (Sporozoa). J. Prot. 9:407-414.

Scholtyseck, E. and H. Mehlhorn. 1970. Recent problems of taxonomy and morphology of coccidia. J. Parasit. 56:307.

Schottelius, M. 1899. Die bedeutung der darmbacterien für die ernährung. I. Arch. Hyg. 34:210-243.

Shapiro, S. K. and W. B. Sarles. 1949. Microorganisms in the intestinal tract of normal chickens. J. Bact. 58:531-544.

Sharma, N. N. and W. M. Reid. 1962. Successful infection of chickens after parenteral inoculation of oocysts of Eimeria sp. J. Parasit. 48 (Suppl.):33.

Smetana, H. 1933. Coccidiosis of the liver in rabbits. I. Experimental study on the excystation of oocysts of Eimeria stiedae. Arch. Path. 15:175-192.

Smith, H. W. 1961. The development of the bacterial flora of the faeces of animals and man: the changes that occur during aging. J. Appl. Bact. 24:235-241.

Smith, H. W. 1965a. Observation of the flora of the alimentary tract of animals and factors affecting its composition. J. Path. Bact. 89:95-122.

Smith, H. W. 1965b. The development of the flora of the alimentary tract in young animals. J. Path. Bact. 90:495-513.

Smith, H. W. and W. E. Crabb. 1961. The faecal bacterial flora of animals and man: its development in the young. J. Path. Bact. 82:53-66.

Smith, H. W. and J. E. T. Jones. 1963. Observations on the alimentary tract and its bacterial flora in healthy and diseased pigs. J. Path. Bact. 86: 387-412.

Smith, H. W. and J. E. T. Jones. 1967. Observations on experimental oral infection with Salmonella dublin in calves and Salmonella choleraesuis in pigs. J. Path. Bact. 93:141-156.

Springer, G. F., R. E. Horton, and M. Forbes. 1959. Origin of antihuman blood group B agglutinins in germ-free chicks. Ann. N. Y. Acad. Sci. 78:272-275.

Springer, W. T. 1968. Histomoniasis: biological aspects of transmission, pathogenesis, and relationships to the indigenous bacterial flora of chickens and turkeys. Doctoral dissertation, University of Georgia, Athens, Georgia.

Springer, W. T., J. Johnson, and W. M. Reid. 1970. Histomoniasis in gnotobiotic chickens and turkeys: biological aspects of the role of bacteria in the etiology. Exp. Parasit. 28:383-392.

Strout, R. G. and C. A. Ouellette. 1969. Gametogony of Eimeria tenella (Coccidia) in cell cultures. Science 165:695-696.

Thorbecke, G. J. 1959. Some histological and functional aspects of lymphoid tissue in germfree animals. I. Morphological studies. Ann. N. Y. Acad. Sci. 78:237-246.

Thorbecke, G. J., H. A. Gordon, B. Wostmann, M. Wagner, and J. A. Reyniers. 1957. Lymphoid tissues and serum gammaglobulin in young germ-free chickens. J. Inf. Dis. 100:237-251.

Timms, L. 1968. Observations on the bacterial flora of the alimentary tract in three age groups of normal chickens. Brit. Vet. J. 124:470-477.

Turk, D. E. and J. F. Stephens. 1967. Gastrointestinal tract disease and zinc absorption: cecal coccidiosis. Poultry Sci. 46:936-943.

Tyzzer, E. E. 1929. Coccidiosis in gallinaceous birds. Am. J. Hyg. 10:269-383.

Tyzzer, E. E., H. Theiler, and E. E. Jones. 1932. Coccidiosis in gallinaceous birds. II. A comparative study of species of Eimeria of the chicken. Am. J. Hyg. 15:319-393.

Vegh, E. 1963. Coccidia resistant to furazolidone. Vet. Bull. 33:117.

Wagenbach, G. E., J. R. Chalfey, and W. C. Burns. 1966. A method for purifying coccidian oocysts employing Clorox and sulphuric acid-dichromate solution. J. Parasit. 52:1222.

Waletzky, E. and C. O. Hughes. 1949-1950. Factors involved in tests for acquired immunity in Eimeria tenella infections of the chicken. Ann. N. Y. Acad. Sci. 52:478-495.

Waletzky, E., C. O. Hughes, and M. C. Brandt. 1949-1950. The anticoccidial activity of nitrophenide. Ann. N. Y. Acad. 52:543-557.

Waxler, S. H. 1941. Changes occurring in the blood and tissues of chickens during coccidiosis and artificial hemorrhage. Am. J. Physiol. 134:19-26.

Weichselbaum, T. E. 1946. An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. Am. J. Clin. Path. 16:40-49.

Weinstein, P. P., W. L. Newton, T. K. Sawyer, and I. Sommerville. 1962. Quoted by W. L. Newton. In W. I. Gay (ed.), Method of Animal Experimentation, Vol. I, 1965. Academic Press, New York, p. 265.

Westcott, R. B. 1971. Experimental infections of gnotobiotic mice with Hematospiroides dubius and Nippostrongylus brasiliensis. J. Parasit. 56:482.

Willingale, J. M. and C. A. E. Briggs. 1955. The normal intestinal flora of the pigs. II. Quantitative bacteriological studies. J. Appl. Bact. 18:284-293.

Wittner, M. and R. M. Rosenbaum. 1970. Role of bacteria in modifying virulence of Entamoeba histolytica. Studies of amebae from axenic cultures. Am. J. Trop. Med. Hyg. 19:755-761.

Wostmann, B. S. 1959. Serum proteins in germ free vertebrates. Ann. N. Y. Acad. Sci. 78:254-260.

Wostmann, B. S. and E. Bruckner-Kardoss. 1959. Development of cecal distention in germ-free baby rats. Am. J. Physiol. 197:1345-1346.

BIOGRAPHICAL SKETCH

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In June, 1953, he enrolled at the Intermediate College, Trivandrum, and passed the interscience examination with distinction in physics and chemistry in March, 1955. From August, 1955, to April, 1959, he attended the Kerala Veterinary College and Research Institute, Trichur, India, and received the degree of Bachelor of Veterinary Science with first class. During his college days he was a member of the soccer, hockey, and field and track teams, and also represented his college in the intercollegiate debate competitions. From July, 1959, to July, 1964, he served as Junior Lecturer at the Kerala Veterinary College and from July, 1966, to September, 1968, he served as scientific officer at the Antibiotics Research Laboratories, Poona, India.

He married the former Jayalakshmy Subramaniam in December, 1965. They have one son, Raja Venkitasubhan.

He is a member of the American Society of Parasitologists, the Southeastern Society of Parasitologists, and the honorary fraternities Alpha Zeta and Phi Sigma.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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